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<p>(54) Title: METHOD OF TREATMENT FOR LUNG DISEASES USING ANTISENSE OLIGONUCLEOTIDES</p> <p>(57) Abstract</p> <p>A method of treating airway disease in a subject in need of such treatment is disclosed. The method comprises topically administering to the subject an antisense oligonucleotide in an amount effective to treat the airway disease, where the antisense oligonucleotide is essentially free of adenosine. Pharmaceutical formulations are also disclosed.</p>		

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METHOD OF TREATMENT FOR LUNG DISEASES USING ANTISENSE OLIGONUCLEOTIDES

This invention was made with Government support under grant RO1CA47217-06 from the National Cancer Institute. The Government has certain rights to this invention.

5

Field of the Invention

This application concerns a method of administering antisense oligonucleotides essentially free of adenosine as a treatment for lung diseases.

Background of the Invention

10

Antisense oligonucleotides have received considerable theoretical consideration as potentially useful pharmacologic agents in human disease. R. Wagner, *Nature* 372, 333-335 (1994). However, practical applications of these molecules in actual models of human disease have been elusive. One important consideration in the pharmacologic application of these molecules is route of administration. Most experiments utilizing antisense oligonucleotides in vivo have involved direct application to limited regions of the brain (see C. Wahlestedt, *Trends in Pharmacological Sciences* 15, 42-46 (1994); J. Lai et al., *Neuroreport* 5, 1049-1052 (1994); K. Standifer et al., *Neuron* 12, 805-810 (1994); A. Akabayashi et al., *Brain Research* 21, 55-61 (1994)), or to spinal fluid (see e.g. L. Tseng et al., *European J. Pharmacol.* 258, R1-3 (1994); R. Raffa et al., *European J. Pharmacol.* 258, R5-7 (1994); F. Gillardon et al., *European J. Neurosci.* 6, 880-884 (1994)). Such applications have limited clinical utility due to their invasive nature.

The systemic administration of antisense oligonucleotides also poses significant problems with respect to pharmacologic application, not the least of which is the difficulty in targeting disease-involved
5 tissues. In contrast, the lung is an excellent potential target for antisense oligonucleotide application since it may be approached noninvasively and in a tissue-specific manner. Additionally, the lung represents an exceptional target for antisense ODN therapeutics as compared to other
10 in vivo target organs or tissues, possibly because the lung is lined with surfactant which consists primarily of cationic lipids, well known to enhance cellular uptake of ODNs in other systems. However, the technology involved in delivering antisense agents to the lung remains
15 relatively undeveloped, and potential problems related to the application of antisense agents to the lung remain unexplored.

Adenosine, a purine which contributes to intermediary metabolism and participates in the
20 regulation of physiological activity, is a recognized neuromodulator. This nucleoside is involved in many local regulatory mechanisms, in particular at synapses in the CNS and at neuroeffector junctions in the periphery. In the CNS adenosine is known to inhibit the release of
25 a variety of neurotransmitters (noradrenaline, serotonin, GABA, acetylcholine, dopamine, glutamate, etc.), to inhibit neurotransmission, depress neuronal firing, induce spinal analgesia, and to possess anxiolytic properties (E.S. Ben-Soreket al., *Archives of Internal*
30 *Medicine* 153, 2701-2702 (1993)). In the heart, adenosine is known to slow atrioventricular (AV) conduction, suppress pacemaker activity, possess antiarrhythmic effects, modulate autonomic control, and to trigger the synthesis and release of prostaglandins. M.K. Church et
35 al., *J. Allergy & Clinical Immunology* 92, 190-194 (1993). It also possesses potent vasodilatory effects and modulates vascular tone. S.T. Holgate et al., *Annals*

of the New York Academy of Sciences 629, 227-236
(1991).

As a therapeutic agent, adenosine has achieved considerable recent success as an antiarrhythmic agent in the treatment of supraventricular tachycardia. See C.G. DeGroff and M.J. Silka, *Journal of Pediatrics* 125, 822-823 (1994); I. Drake et al., *Human and Exp. Toxicol.* 13, 263-265 (1994). However, many adverse effects of adenosine treatment have been reported in the literature. See, e.g., A. Aggarwal, et al., *Anesthesiology* 79, 1132-1135 (1993); K.K. Burkhardt, *American J. Emergency Med.* 11, 249-250 (1993); S.K. Srinivasan and P.J. Iversen, *J. Clin. Lab. Analysis* 9, 129-137 (1995); C.A. Stein et al., *Pharmacology & Therapeutics* 52, 365-384 (1991); B.B. Fredholm et al., *Pharmacological Reviews* 46, 143-156 (1994); H. Saito, et al., *Blood* 66, 1233-1240 (1985). In particular, asthmatic individuals show an extreme sensitivity to adenosine and adenosine monophosphate. See, J.H. Butterfield et al., *Leukemia Res.* 12, 345-355 (1988); *CLONETICS: Normal Human Cell Systems Manual* (1995); R.W. Wagner, *Nature* 372, 333-335 (1994). Serious, near-fatal induction of bronchospasm has occurred in asthmatic individuals administered adenosine for supraventricular tachycardia. See, S. Tabor, in: *Current Protocols in Molecular Biology*, Vol. 1, Section 3.10.2 (John Wiley & Sons, 1987); J.H. Weiss, Id., at Section 6.2.2.

Similarly, asthmatic rabbits produced using the dust mite allergic rabbit model of human asthma also were shown to respond to aerosolized adenosine with marked bronchoconstriction, while non asthmatic rabbits showed no response. S. Ali et al., *Agents Actions* 37, 165-176 (1992). Recent work using this model system has suggested that adenosine-induced bronchoconstriction and bronchial hyperresponsiveness in asthma are mediated primarily through the stimulation of adenosine receptors. S. Ali et

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al., *J. Pharmacol. Exp. Ther.* 268, 1328-1334 (1994); S. Ali et al., *Am. J. Physiol* 266, L271-277 (1994).

Accordingly, adenosine is contraindicated in the lungs of asthmatics (who represent 10% of the adult and 15% of the pediatric population in the United States). Since antisense ODNs are typically composed of all four base pairs, adenine, guanine, cytosine and thymidine, their breakdown products will produce free deoxyadenosine monophosphate in these hyperresponsive airways. Deoxyadenosine monophosphate differs from adenosine monophosphate only by the loss of an oxygen atom on the 3' carbon of the sugar moiety.

Summary of the Invention

A first aspect of the present invention is a method of treating airway disease in a subject in need of such treatment. The method comprises administering an antisense oligonucleotide essentially free of adenosine to the lungs of the subject in an amount effective to treat the airway disease.

A second aspect of the present invention is a pharmaceutical composition, comprising, together in a pharmaceutically acceptable carrier, an antisense oligonucleotide essentially free of adenosine in an amount effective to treat an airway disease.

A third aspect of the present invention is the use of an antisense oligonucleotide essentially free of adenosine as given above for the preparation of a medicament for treating airway disease in a subject in need of such treatment.

Brief Description of the Drawings

Figures 1-4 demonstrate that antisense oligonucleotides can be utilized as effective agents in the treatment or prevention of airway diseases.

Figure 1 illustrates the effects of A₁ adenosine receptor antisense oligonucleotides and mismatch control

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antisense oligonucleotides on the dynamic compliance of the bronchial airway in a rabbit model. Figure 2 illustrates the specificity of A₁ adenosine receptor antisense oligonucleotides as indicated by the A₁ and A₂ adenosine receptor number present in A₁ adenosine receptor antisense oligonucleotide-treated airway tissue.

Figure 3 is a graphical representation illustrating that aerosolized deoxyadenosine monophosphate is a potent bronchoconstrictor in asthmatic pathways of allergic rabbits. Further, the figure shows that the effect of deoxyadenosine monophosphate is equipotent to that observed for adenosine monophosphate.

Figure 4 is a graphical representation illustrating that bronchoconstrictor effects occur with aerosolized phosphorothioate oligodeoxynucleotides containing adenosine, but not with oligodeoxynucleotides that are free of adenosine.

Detailed Description of the Invention

Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with 37 CFR §1.822 and established usage. See, e.g., Patent In User Manual, 99-102 (Nov. 1990) (U.S. Patent and Trademark Office, Office of the Assistant Commissioner for Patents, Washington, D.C. 20231); U.S. Patent No. 4,871,670 to Hudson et al. at Col. 3 - lines 20-43 (applicants specifically intend that the disclosure of this and all other patent references cited herein be incorporated herein by reference).

The method of the present invention may be used to treat airway disease in a subject for any reason, with the intention that adenosine content of antisense compounds be eliminated or reduced so as to prevent its

liberation upon antisense degradation. Such liberation may cause serious, even life-threatening, bronchoconstriction in patients with hyperreactive airways. Examples of airway diseases that may be treated
5 by the method of the present invention include cystic fibrosis, asthma, chronic obstructive pulmonary disease, bronchitis, and other airway diseases characterized by an inflammatory response.

Antisense oligonucleotides to the A₁ and A₂ receptors are shown to be effective in the downregulation of A₁ or A₂ in the cell. One novel feature of this treatment, as compared to traditional treatments for adenosine-induced bronchoconstriction, is that administration is direct to the lungs. Additionally, a
15 receptor protein itself is reduced in amount, rather than merely interacting with a drug, and toxicity is reduced. Other proteins that may be targeted with antisense agents for the treatment of lung conditions include, but are not limited to: human A_{2a} adenosine receptor, human A_{2b}
20 adenosine receptor, human IgE receptor β , human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil
25 derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion molecule-1 (ICAM-1), human vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor,
30 human IL-3, human IL-4, human IL-5, human IL-6, human IL-8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-
35 alpha, human muscarinic acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor α , human

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leukotriene C4 synthase, human major basic protein, and human endothelin 1. In these latter targets, and in target genes in general, it is particularly imperative to eliminate or reduce the adenosine content of the
5 corresponding antisense oligonucleotide to prevent their breakdown products from liberating adenosine.

As used herein, the term "treat" or "treating" a lung disease refers to a treatment which decreases the likelihood that the subject administered such treatment
10 will manifest symptoms of the lung disease. The term "downregulate" refers to inducing a decrease in production, secretion or availability (and thus a decrease in concentration) of the targeted intracellular protein.

15 The present invention is concerned primarily with the treatment of human subjects but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes. Targeted proteins are preferably mammalian and more preferably of
20 the same species as the subject being treated.

In general, "antisense" refers to the use of small, synthetic oligonucleotides, resembling single-stranded DNA, to inhibit gene expression by inhibiting the function of the target messenger RNA (mRNA).
25 Milligan, J.F. et al., *J. Med. Chem.* 36(14), 1923-1937 (1993). In the present invention, inhibition of gene expression of the A₁ or A₂ adenosine receptor is desired. Gene expression is inhibited through hybridization to coding (sense) sequences in a specific messenger RNA
30 (mRNA) target by hydrogen bonding according to Watson-Crick base pairing rules. The mechanism of antisense inhibition is that the exogenously applied oligonucleotides decrease the mRNA or protein levels of the target gene or cause changes in the growth
35 characteristics or shapes of the cells. *Id.* See also Helene, C. and Toulme, J., *Biochim. Biophys. Acta* 1049, 99-125 (1990); Cohen, J.S., Ed., *Oligodeoxynucleotides as*

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Antisense Inhibitors of Gene Expression; CRC Press: Boca Raton, FL (1987).

As used herein, "antisense oligonucleotide" is defined as a short sequence of synthetic nucleotides that
5 (1) hybridizes to any coding sequence in an mRNA which codes for the targeted protein, according to hybridization conditions described below, and (2) upon hybridization causes a decrease in gene expression of the targeted protein.

10 The mRNA sequence of the A₁ or A₃ adenosine receptor is derived from the DNA base sequence of the gene expressing either the A₁ or A₃ adenosine receptor. The sequence of the genomic human A₁ adenosine receptor is known and is disclosed in U.S. Patent No. 5,320,963 to G.
15 Stiles et al. The A₃ adenosine receptor has been cloned, sequenced and expressed in rat (see F. Zhou et al., *Proc. Nat'l Acad. Sci. USA* 89:7432 (1992)) and human (see M.A. Jacobson et al., U.K. Patent Application No. 9304582.1 (1993)). Thus, antisense oligonucleotides that
20 downregulate the production of the A₁ or A₃ adenosine receptor may be produced in accordance with standard techniques.

One aspect of this invention is an antisense oligonucleotide having a sequence capable of binding
25 specifically with any sequence of an mRNA molecule which encodes an airway disease-associated protein so as to prevent translation of the mRNA molecule.

Chemical analogs of oligonucleotides (e.g., oligonucleotides in which the phosphodiester bonds have
30 been modified, e.g., to the methylphosphonate, the phosphotriester, the phosphorothioate, the phosphorodithioate, or the phosphoramidate, so as to render the oligonucleotide more stable in vivo) are also an aspect of the present invention. The naturally
35 occurring phosphodiester linkages in oligonucleotides are susceptible to degradation by endogenously occurring cellular nucleases, while many analogous linkages are

highly resistant to nuclease degradation. See Milligan et al., and Cohen, J.S., *supra*. Protection from degradation can be achieved by use of a "3'-end cap" strategy by which nuclease-resistant linkages are substituted for phosphodiester linkages at the 3' end of the oligonucleotide. See Tidd, D.M. and Warenus, H.M., *Br. J. Cancer* 60, 343-350 (1989); Shaw, J.P. et al., *Nucleic Acids Res.* 19, 747-750 (1991). Phosphoramidates, phosphorothioates, and methylphosphonate linkages all function adequately in this manner. More extensive modification of the phosphodiester backbone has been shown to impart stability and may allow for enhanced affinity and increased cellular permeation of oligonucleotides. See Milligan, et al., *supra*. Many different chemical strategies have been employed to replace the entire phosphodiester backbone with novel linkages. *Id.* Backbone analogues include phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, boranophosphate, phosphotriester, formacetal, 3'-thioformacetal, 5'-thioformacetal, 5'-thioether, carbonate, 5'-N-carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methylimino) (MMI) or methyleneoxy(methylimino) (MOMI) linkages. Phosphorothioate and methylphosphonate-modified oligonucleotides are particularly preferred due to their availability through automated oligonucleotide synthesis. *Id.* Where appropriate, the antisense oligonucleotides may be administered in the form of their pharmaceutically acceptable salts.

Antisense oligonucleotides may be of any suitable length (e.g., from about 10 to 60 nucleotides in length), depending on the particular target being bound and the mode of delivery thereof. Preferably the antisense oligonucleotide is directed to an mRNA region containing a junction between intron and exon. Where the antisense oligonucleotide is directed to an intron/exon

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junction, it may either entirely overlie the junction or may be sufficiently close to the junction to inhibit splicing out of the intervening exon during processing of precursor mRNA to mature mRNA (e.g., with the 3' or 5' terminus of the antisense oligonucleotide being is positioned within about, for example, 10, 5, 3, or 2 nucleotides of the intron/exon junction).

When practicing the present invention, the antisense nucleotides administered may be related in origin to the species to which it is administered. When treating humans, human antisense may be used if desired.

Pharmaceutical compositions comprising an antisense oligonucleotide as given above effective to reduce expression of an A_1 or A_2 adenosine receptor by passing through a cell membrane and binding specifically with mRNA encoding an A_1 or A_2 adenosine receptor in the cell so as to prevent its translation are another aspect of the present invention. Such compositions are provided in a suitable pharmaceutically acceptable carrier (e.g., sterile pyrogen-free saline solution). The antisense oligonucleotides may be formulated with a hydrophobic carrier capable of passing through a cell membrane (e.g., in a liposome, with the liposomes carried in a pharmaceutically acceptable aqueous carrier). The oligonucleotides may also be coupled to a substance which inactivates mRNA, such as a ribozyme. Such oligonucleotides may be administered to a subject to inhibit the activation of A_1 or A_2 adenosine receptors, which subject is in need of such treatment for any of the reasons discussed herein. Furthermore, the pharmaceutical formulation may also contain chimeric molecules comprising antisense oligonucleotides attached to molecules which are known to be internalized by cells. These oligonucleotide conjugates utilize cellular uptake pathways to increase cellular concentrations of oligonucleotides. Examples of macromolecules used in this manner include transferrin, asialoglycoprotein

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(bound to oligonucleotides via polylysine) and streptavidin.

In the pharmaceutical formulation the antisense compound may be contained within a lipid particle or vesicle, such as a liposome or microcrystal. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein. Positively charged lipids such as N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl-ammoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles.

The preparation of such lipid particles is well known. See, e.g., U.S. Patent Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.; 4,921,757 to Wheatley et al.; etc.

Subjects may be administered the active composition by any means which transports the antisense nucleotide composition to the lung. The antisense compounds disclosed herein may be administered to the lungs of a patient by any suitable means, but are preferably administered by generating an aerosol comprised of respirable particles, the respirable particles comprised of the antisense compound, which particles the subject inhales. The respirable particles may be liquid or solid. The particles may optionally contain other therapeutic ingredients.

Particles comprised of antisense compound for practicing the present invention should include particles of respirable size: that is, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about .5 to 10 microns in size are respirable. Particles of non-respirable size which are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is preferably

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minimized. For nasal administration, a particle size in the range of 10-500 μm is preferred to ensure retention in the nasal cavity.

Liquid pharmaceutical compositions of active compound for producing an aerosol can be prepared by combining the antisense compound with a suitable vehicle, such as sterile pyrogen free water. Other therapeutic compounds may optionally be included.

Solid particulate compositions containing respirable dry particles of micronized antisense compound may be prepared by grinding dry antisense compound with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprised of the antisense compound may optionally contain a dispersant which serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the antisense compound in any suitable ratio (e.g., a 1 to 1 ratio by weight). Again, other therapeutic compounds may also be included.

The dosage of the antisense compound administered will depend upon the disease being treated, the condition of the subject, the particular formulation, the route of administration, the timing of administration to a subject, etc. In general, intracellular concentrations of the oligonucleotide of from .05 to 50 μM , or more particularly .2 to 5 μM , are desired. For administration to a subject such as a human, a dosage of from about .01, .1, or 1 mg/Kg up to 50, 100, or 150 mg/Kg or more is typically employed. Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. Administration of the antisense compounds may be carried out therapeutically (i.e., as a rescue treatment) or prophylactically.

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Aerosols of liquid particles comprising the antisense compound may be produced by any suitable means, such as with a nebulizer. See, e.g., U.S. Patent No. 4,501,729. Nebulizers are commercially available devices which transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. the carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

Aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles which are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn

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through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 μ l, to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants, such as oleic acid or sorbitan trioleate, antioxidants and suitable flavoring agents.

The aerosol, whether formed from solid or liquid particles, may be produced by the aerosol generator at a rate of from about 10 to 150 liters per minute, more preferably from about 30 to 150 liters per minute, and most preferably about 60 liters per minute. Aerosols containing greater amounts of medicament may be administered more rapidly.

The following examples are provided to illustrate the present invention, and should not be construed as limiting thereon. In these examples, μ M means micromolar, mL means milliliters, μ m means micrometers, mm means millimeters, cm means centimeters, $^{\circ}$ C means degrees Celsius, μ g means micrograms, mg means

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milligrams, g means grams, kg means kilograms, M means molar, and h means hours.

EXAMPLE 1

Design and synthesis of antisense oligonucleotides

5 The design of antisense oligonucleotides against the A₁ and A₂ adenosine receptors may require the solution of the complex secondary structure of the target A₁ receptor mRNA and the target A₂ receptor mRNA. After generating this structure, antisense nucleotides are
10 designed which target regions of mRNA which might be construed to confer functional activity or stability to the mRNA and which optimally may overlap the initiation codon. Other target sites are readily usable. As a demonstration of specificity of the antisense effect,
15 other oligonucleotides not totally complementary to the target mRNA, but containing identical nucleotide compositions on a w/w basis, are included as controls in antisense experiments.

 Adenosine A₁ receptor mRNA secondary structure
20 was analyzed and used as described above to design a phosphorothioate antisense oligonucleotide. The antisense oligonucleotide which was synthesized was designated HAdA1AS and had the following sequence:

5'-GAT GGA GGG CGG CAT GGC GGG-3' (SEQ ID NO:1)

25 As a control, a mismatched phosphorothioate antisense nucleotide designated HAdA1MM was synthesized with the following sequence:

5'-GTA GCA GGC GGG GAT GGC GGC-3' (SEQ ID NO:2)

Each oligonucleotide had identical base content and
30 general sequence structure. Homology searches in GENBANK (release 85.0) and EMBL (release 40.0) indicated that the antisense oligonucleotide was specific for the human and rabbit adenosine A₁ receptor genes, and that the

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mismatched control was not a candidate for hybridization with any known gene sequence.

Adenosine A₁ receptor mRNA secondary structure was similarly analyzed and used as described above to design two phosphorothioate antisense oligonucleotides. The first antisense oligonucleotide (HAdA3AS1) synthesized had the following sequence:

5'-GTT GTT GGG CAT CTT GCC-3' (SEQ ID NO:3)

As a control, a mismatched phosphorothioate antisense oligonucleotide (HAdA3MM1) was synthesized, having the following sequence:

5'-GTA CTT GCG GAT CTA GGC-3' (SEQ ID NO:4)

A second phosphorothioate antisense oligonucleotide (HAdA3AS2) was also designed and synthesized, having the following sequence:

5'-GTG GGC CTA GCT CTC GCC-3' (SEQ ID NO:5)

Its control oligonucleotide (HAdA3MM2) had the sequence:

5'-GTC GGG GTA CCT GTC GGC-3' (SEQ ID NO:6)

Phosphorothioate oligonucleotides were synthesized on an Applied Biosystems Model 396 Oligonucleotide Synthesizer, and purified using NENSORB chromatography (DuPont, MD).

EXAMPLE 2

Testing of A₁-Adenosine Receptor

Antisense Oligonucleotides in vitro

The antisense oligonucleotide against the human A₁ receptor (SEQ ID NO:1) described above was tested for

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efficacy in an in vitro model utilizing lung adenocarcinoma cells HTB-54. HTB-54 lung adenocarcinoma cells were demonstrated to express the A₁ adenosine receptor using standard northern blotting procedures and
5 receptor probes designed and synthesized in the laboratory.

HTB-54 human lung adenocarcinoma cells (106/100 mm tissue culture dish) were exposed to 5.0 μ M HAdA1AS or HAdA1MM for 24 hours, with a fresh change of media and
10 oligonucleotides after 12 hours of incubation. Following 24 hour exposure to the oligonucleotides, cells were harvested and their RNA extracted by standard procedures. A 21-mer probe corresponding to the region of mRNA targeted by the antisense (and therefore having the same
15 sequence as the antisense, but not phosphorothioated) was synthesized and used to probe northern blots of RNA prepared from HAdA1AS-treated, HAdA1MM-treated and non-treated HTB-54 cells. These blots showed clearly that HAdA1AS but not HAdA1MM effectively reduced human
20 adenosine receptor mRNA by >50%. This result showed that HAdA1AS is a good candidate for an anti-asthma drug since it depletes intracellular mRNA for the adenosine A₁ receptor, which is involved in asthma.

EXAMPLE 3

25 Efficacy of A₁-Adenosine Receptor Antisense Oligonucleotides in vivo

A fortuitous homology between the rabbit and human DNA sequences within the adenosine A₁ gene overlapping the initiation codon permitted the use of the
30 phosphorothioate antisense oligonucleotides initially designed for use against the human adenosine A₁ receptor in a rabbit model.

Neonatal New Zealand white Pasteurella-free rabbits were immunized intraperitoneally within 24 hours
35 of birth with 312 antigen units/mL house dustmite (*D. farinae*) extract (Berkeley Biologicals, Berkeley, CA),

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mixed with 10% kaolin. Immunizations were repeated weekly for the first month and then biweekly for the next 2 months. At 3-4 months of age, eight sensitized rabbits were anesthetized and relaxed with a mixture of ketamine 5 hydrochloride (44 mg/kg) and acepromazine maleate (0.4 mg/kg) administered intramuscularly.

The rabbits were then laid supine in a comfortable position on a small molded, padded animal board and intubated with a 4.0-mm intratracheal tube 10 (Mallinkrodt, Inc., Glens Falls, NY). A polyethylene catheter of external diameter 2.4 mm with an attached latex balloon was passed into the esophagus and maintained at the same distance (approximately 16 cm) from the mouth throughout the experiments. The 15 intratracheal tube was attached to a heated Fleisch pneumotachograph (size 00; DOM Medical, Richmond, VA), and flow was measured using a Validyne differential pressure transducer (Model DP-45161927; Validyne Engineering Corp., Northridge, CA) driven by a Gould 20 carrier amplifier (Model 11-4113; Gould Electronic, Cleveland, OH). The esophageal balloon was attached to one side of the differential pressure transducer, and the outflow of the intratracheal tube was connected to the opposite side of the pressure transducer to allow 25 recording of transpulmonary pressure. Flow was integrated to give a continuous tidal volume, and measurements of total lung resistance (RL) and dynamic compliance (Cdyn) were calculated at isovolumetric and flow zero points, respectively, using an automated 30 respiratory analyzer (Model 6; Buxco, Sharon, CT).

Animals were randomized and on Day 1 pretreatment values for PC50 were obtained for aerosolized adenosine. Antisense (HAdA1AS) or mismatched control (HAdA1MM) oligonucleotides were dissolved in 35 sterile physiological saline at a concentration of 5000 ug (5 mg) per 1.0 ml. Animals were subsequently administered the aerosolized antisense or mismatch

oligonucleotide via the intratracheal tube (approximately 5000 μ g in a volume of 1.0 ml), twice daily for two days.

Aerosols of either saline, adenosine, or antisense or mismatch oligonucleotides were generated by an ultrasonic nebulizer (DeVilbiliss, Somerset, PA), producing aerosol droplets 80% of which were smaller than 5 μ m in diameter.

In the first arm of the experiment, four randomly selected allergic rabbits were administered antisense oligonucleotide and four the mismatched control oligonucleotide. On the morning of the third day, PC50 values (the concentration of aerosolized adenosine in mg/ml required to reduce the dynamic compliance of the bronchial airway 50% from the baseline value) were obtained and compared to PC50 values obtained for these animals prior to exposure to oligonucleotide.

Following a 1 week interval, animals were crossed over, with those previously administered mismatch control oligonucleotide now administered antisense oligonucleotide, and those previously treated with antisense oligonucleotide now administered mismatch control oligonucleotide. Treatment methods and measurements were identical to those employed in the first arm of the experiment. It should be noted that in six of the eight animals treated with antisense oligonucleotide, adenosine-induced bronchoconstriction could not be obtained up to the limit of solubility of adenosine, 20 mg/ml. For the purpose of calculation, PC50 values for these animals were set at 20 mg/ml. The values given therefore represent a minimum figure for antisense effectiveness. Actual effectiveness was higher. The results of this experiment are illustrated in both Figure 1 and Table 1.

TABLE 1. EFFECTS OF ADENOSINE A₁ RECEPTOR ANTISENSE OLIGONUCLEOTIDE UPON PC50 VALUES IN ASTHMATIC RABBITS.

Mismatch Control		A ₁ receptor Antisense oligonucleotide	
Pre oligonucleotide	Post oligonucleotide	Pre oligonucleotide	Post oligonucleotide
3.56 ± 1.02	5.16 ± 1.93	2.36 ± 0.68	>19.5 ± 0.34**

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. **Significantly different from all other groups, P < 0.01.

10 In both arms of the experiment, animals
receiving the antisense oligonucleotide showed an order
of magnitude increase in the dose of aerosolized
adenosine required to reduce dynamic compliance of the
lung by 50%. No effect of the mismatched control
15 oligonucleotide upon PC50 values was observed. No
toxicity was observed in any animal receiving either
antisense or control inhaled oligonucleotide.

These results show clearly that the lung has
exceptional potential as a target for antisense
20 oligonucleotide-based therapeutic intervention in lung
disease. They further show, in a model system which
closely resembles human asthma, that downregulation of
the adenosine A₁ receptor largely eliminates adenosine-
induced bronchoconstriction in asthmatic airways.
25 Bronchial hyperresponsiveness in the allergic rabbit
model of human asthma is an excellent endpoint for
antisense intervention since the tissues involved in this
response lie near to the point of contact with
aerosolized oligonucleotides, and the model closely
30 simulates an important human disease.

EXAMPLE 4

Specificity of A₁-adenosine receptor
Antisense oligonucleotide

At the conclusion of the crossover experiment
5 of Example 3, airway muscle from all rabbits was
quantitatively analyzed for adenosine A₁ receptor number.
As a control for the specificity of the antisense
oligonucleotide, adenosine A₂ receptors, which should not
have been affected, were also quantified.

10 Airway smooth muscle tissue was dissected from
each rabbit and a membrane fraction prepared according to
described methods (J. Kleinstein and H. Glossmann,
Naunyn-Schmiedeberg's Arch. Pharmacol. 305, 191-200
(1978), with slight modifications. Crude plasma membrane
15 preparations were stored at - 70°C until the time of
assay. Protein content was determined by the method of
Bradford (M. Bradford, Anal. Biochem. 72, 240-254
(1976)). Frozen plasma membranes were thawed at room
temperature and were incubated with 0.2 U/ml adenosine
20 deaminase for 30 minutes at 37°C to remove endogenous
adenosine. The binding of [³H]DPCPX (A₁ receptor-
specific) or [³H]CGS-21680 (A₂ receptor-specific) was
measured as previously described. S. Ali et al., J.
Pharmacol. Exp. Ther. 268, 1328-1334 (1994); S. Ali et
25 al., Am. J. Physiol 266, L271-277 (1994).

As illustrated in both Figure 2 and Table 2,
animals treated with adenosine A₁ antisense
oligonucleotide in the crossover experiment had a nearly
75% decrease in A₁ receptor number compared to controls,
30 as assayed by specific binding of the A₁-specific
antagonist DPCPX. There was no change in adenosine A₂
receptor number, as assayed by specific binding of the A₂
receptor-specific agonist 2-[p-(2-carboxyethyl)-
phenethylamino]-5'-(N-ethylcarboxamido) adenosine (CGS-
35 21680).

TABLE 2. SPECIFICITY OF ACTION OF ADENOSINE A₁ RECEPTOR ANTISENSE OLIGONUCLEOTIDE.

	Mismatch Control oligonucleotide	A ₁ Antisense oligonucleotide
5 A ₁ -Specific Binding	1105 ± 48**	293 ± 18
A ₂ -Specific Binding	302 ± 22	442 ± 171

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. **Significantly different from mismatch control, P < 0.01.

10 The above demonstrates the effectiveness of antisense
oligonucleotides in treating airway diseases. Since the
antisense oligonucleotides described above eliminate the
receptor systems responsible for adenosine-mediated
15 bronchoconstriction, it may be less imperative to
eliminate adenosine from them. However, it would be
preferable to eliminate adenosine from even these
oligonucleotides. Examples of such adenosine-free
oligonucleotides are provided below in Example 5.

EXAMPLE 5

20 The method of the present invention is also
practiced with the following antisense oligonucleotides
targeted to their corresponding proteins, in essentially
the same manner as given above, for the treatment of
various conditions in the lungs. Described below is a
25 series of antisense oligonucleotides targetting the mRNA
of proteins involved in inflammation. Adenosine has
been eliminated from their nucleotide content to prevent
its liberation during degradation.

In the following, the first sequence provided
30 after the name of the targeted inflammation-involved
protein is the antisense sequence that targets the
initiation codon, wherein the naturally-occurring
adenosine is substituted by one of the following: (1) a
universal base that is not adenosine; (2) a adenosine
35 analog that lacks the ability to bind to the adenosine A1

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and/or A3 receptors; or (3) a "spacer." Any one of these three is represented in the sequence as the letter "B," recognized by the IUPAC-IUB Nomenclature Commission as "not-A." See *Patentin User Manual*, p.99 (November 1990).

5 Listed following the antisense sequence targeted against the initiation codon are additional antisense oligonucleotide sequences directed against other portions of the mRNA of the targeted protein. These additional sequences are the "des-adenosine antisense sequences," in
10 that they do not contain adenosine within the sequence.

Fragments of the following sequences that are at least ten, and more preferably at least twelve, nucleotides in length are also an aspect of the present invention and are useful in carrying out the present
15 invention. Fragments set forth below that span multiple lines of text indicate "5'" at the beginning thereof, and "-3'" at the end thereof.

Human A1 adenosine receptor:

20 5'-GGC GGC CTG GBB BGC TGB GBT GGB GGG CGG CBT
GGC GGG CBC BGG CTG GGC-3'

des-adenosine antisense sequences:
TTT TCC TTC CTT TGT CTC TCT TC

GCT CCC GGC TGC CTG

CTC GGC CGT GCG GCT CTG TCG CTC CCG GT

25 CCG CCG CCC TCC GGG GGG TC

TGC TGC CGT TGG CTG CCC

CTT CTG CGG GTC GCC GG

TGC TGG GCT TGT GGC

GGC CTC TCT TCT GGG

30 CCT GGT CCC TCC GT

GGT GGC TCC TCT GC

GCT TGG TCC TGG GGC TGC

TGC TCT CCT CTC CTT

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Human A2a adenosine receptor:

GTBCBCCGBGGBGCCCBTGBTGGGCBTGCCBCBGBCBGBCBGGC

des-adenosine antisense sequences:

5 HSA2ARECAS1: TGC TTT TCT TTT CTG GGC CTC (SEQ ID NO:7)

HSA2ARECAS2: TGT GGT CTG TTT TTT TCT G

HSA2ARECAS3: GCC CTG CTG GGG CGC TCT CC

HSA2ARECAS4: GCC GCC CGC CTG GCT CCC

10 HSA2ARECAS5: GGB GCC CBT GBT GGG CBT GCC

HSA2ARECAS6: GTG GTT CTT GCC CTC CTT TGG CTG

HSA2ARECAS7: CCG TGC CCG CTC CCC GGC

HSA2ARECAS8: CTC CTG GCG GGT GGC CGT TG

HSA2ARECAS9: GGC CCG TGT TCC CCT GGG

15 HSA2ARECAS10: GCC TGG GGC TCC CTT CTC TC

HSA2ARECAS11: GCC CTT CTT GCT GGG CCT C

HSA2ARECAS12: TGC TGC TGC TGG TGC TGT GGC CCCC

Human A2b adenosine receptor:

5'-BCBGC GCGTCCTGTGTCTCCBGCBCBTGGCC

GGGCCBGTGGGCCCC-3'

20 *des-adenosine antisense sequences:*

HSA2BRECAS1: 5'-GGC GCC GTG CCG CGT CTT GGT GGC

GGC GG-3' (SEQ ID NO:8)

HSA2BRECAS2: 5'-GTT CGC GCC CGC GCG GGG CCC CTC

CGG TCC-3'

25 HSA2BRECAS3: 5'-TTG GCC CGC GCG CCC GCC CGT CTC

GGG CTG GGC GG-3'

HSA2BRECAS4: CCG GTC GGG GCC CCC CGC GGC C

HSA2BRECAS5: 5'-GCC TCG GGG CTG GGG CGC TGG TGG

CCG GG-3'

30 HSA2BRECAS6: CCG CGC CTC CGC CTG CCG CTT CTG

HSA2BRECAS7: GCT GGG CCC CGG GCG CCC CCT

HSA2BRECAS8: CCC CTC TTG CTC GGG TCC CCG TG

Human A3 adenosine receptor

35 5'-BCB GBG CBG TGC TGT TGT TGG GCB TCT TGC CTT

CCC BGG G-3'

des-adenosine antisense oligonucleotides:

CCC TTT TCT GGT GGG GTG

GTG CTG TTG TTG GGC

TTT CTT CTG TTC CC

40 Human IgE receptor β :

5'-BTTTGCTCTCCTBTTBCTTTCTGTGTCCBTTT

CBTTBBCCGBGCTGT-3'

des-adenosine antisense sequences:

45 HUMIgE β rAS1: TTT CCC CTG GGT CTT CC (SEQ ID NO:9)

HUMIgE β rAS2: CTC CTG CTC TTT TTT C

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Human Fc-epsilon receptor CD23 antigen (IgE receptor):

5'-TCTCTGBBTBTGBCCTTCCTCCBTGGCGGTCCTGCTT
GGBTTCTCCCGB-3'

des-adenosine antisense sequences:

5 HUMIgErCD23AS1: GCC TGT GTC TGT CCT CCT (SEQ ID NO:10)
HUMIgErCD23AS2: GCT TCG TTC CTC TCG TTC
HUMIgErCD23AS3: CTG CTT GGT GCC CTT GCC G
HUMIgErCD23AS4: GTC CTG CTC CTC CGG GCT GTG G
10 HUMIgErCD23AS5: 5'-GTC GTG GCC CTG GCT CCG
GCTGGT GGG CTC CCC TGG-3'
HUMIgErCD23AS6: CCT TCG CTG GCT GGC GGC GTG C
HUMIgErCD23AS7: GGG TCT TGC TCT GGG CCT GGC TGT
HUMIgErCD23AS8: GGC CGT GGT TGG GGG TCT TC
15 HUMIgErCD23AS9: GCT GCC TCC GTT TGG GTG GC

Human IgE receptor, α subunit:

5'-BCBGTBGBGTBGGGGBTTCBTTGGCBGGBGCCBTC
TTCTTCBTGGBCTCC-3'

and

20 5'-TTC BBG GBG BCC TTB GGT TTC TGB GGG BCT GCT
BBC BCG CCB TCT GGB GC-3'

des-adenosine antisense sequences:

HUMIgEr α AS1: GCCTTTCCTGGTTCTCTT (SEQ ID NO:11)
GTT GTT TTT GGG GTT TGG CTT

25 Human IgE receptor, Fc epsilon R:

5'-GBT CTC TGB BTB TTGB CCT TCC BTG GCG GTC CTG
CTT GGB-3'

des-adenosine antisense sequences:

30 HSJGEBFRAS1: GCC TGT GTC TGT CCT CCT (SEQ ID NO:12)
HSJGEBFRAS2: GCT TCG TTC CTC TCG TTC
HSJGEBFRAS3: CTG CTT GGT GCC CTT GCC G
HSJGEBFRAS4: GTC CTG CTC CTC CGG GCT GTG G
HSJGEBFRAS5: 5'-GTC CTC GCC CTG GCT CCG GCT GGT
35 GGG CTC CCC TGG-3'
HSJGEBFRAS6: CCT TCG CTG GCT GGC GGC GTG C
HSJGEBFRAS7: CCC BGB BCG BGB CCC GGB CCG BCB
HSJGEBFRAS8: GGC CGT GGT TGG GGG TCT TC
HSJGEBFRAS9: GCT GCC TCC GTT TGG GTG GC

40 Human histidine decarboxylase:

5'-CTC TGT CCC TCT CTC TCT GTB CTC CTC BGG CTC
CBT CBT CTC CCT TGG GC-3'

des-adenosine antisense sequences:

45 HUMHDCAS1: TCT CCC TTG GGC TCT GGC TCC TTC TC
(SEQ ID NO:13)

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HUMHDCAS2: TCT CTC TCC CTC TCT CTC TGT
 HUMHDCAS3: CGCCTCCGCCCTGGCTGCTGGGGTGGTGGTGC
 HUMHDCAS4: TTT TGT TCT TCC TTG CTG CC
 HUMHDCAS5: GCC CCG CTG CTT GTC TTC CTC G

5 Human beta tryptase:

5'-GGG CCT GGC CTG GGG CBG GGG CCG CGT BGG CGC
 GGC TCG CCB GGB CGG GCB GCG CCB GCB GCB GCB GBT
 TCB GCB TCC TGG-3'

des-adenosine antisense sequences:

10 HUMBTRYPAS1: CTTGCTCCTGGGGGCCTCCTG (SEQ ID
 NO:14)
 HUMBTRYPAS2: GTC CCT CCG GGT GTT CCC GGC

Human tryptase-I:

15 5'-CCT GGB CTG GGG CBG GGG CCG CGT BGG CGC GGC
 TCG CCB GGB CGG GCB GCG CCB GCB GCB GCB GGC TCB
 GCB TCC TGG CCB CGG BBT TCC-3'

des-adenosine antisense sequences:

HUMTRYAS1: CTTGCTCCTGGGGGCCTCCTG (SEQ ID NO:15)
 HUMTRYAS2: GTC CCT CTG GCT G TT CCC GGC

20 Human prostaglandin D synthase:

5'-CCC CBG CBG GBC CBG TCC CBT CCB CBG CGT GTG
 BTG BGT BGC CBT TCT CCT GCB GCC GBG-3'

des-adenosine antisense sequences:

25 HUMPROSYNAS1:GGTGTGCGGGGCCTGGTGCC(SEQ ID NO:16)
 HUMPROSYNAS 2: CCT GGG CCT CGG GTG CTG CCT GT
 HUMPROSYNAS 3: GCG CTG CCT TCT TCT CCT GG
 HUMPROSYNAS 4: 5'-GTC CTC GCC GGG GCC CTT GCT
 GCC CTG GCT GT -3'
 HUMPROSYNAS 5: GCC CTG GGG GTC TGG GTT CGGCTGT

30 Human cyclooxygenase-2:

5'-TGB GCG CCB GGB CCG CGC BCB GCB GCB GGG CGC
 GGG CGB GCB TCG CBG CGG CGG GCB GGG-3'

des-adenosine antisense sequences:

35 HUMCYCLOXAS1: GGGCGCGGGCGBGCBTCGC(SEQ ID NO:17)
 HUMCYCLOXAS2: TTT GGG CTT TTC TCC TTT GGT T

Human eosinophil cationic protein:

5'-CBG BCB BBT TTG GGB BGT GBB CBG TTT TGG BBC
 CBT GTT TCC CBG TCT CTG BGC TGT GGC-3'

des-adenosine antisense sequences:

40 HSECPAS1: CCTCCTTCC TGG TCT GTC TGC (SEQ ID
 NO:18)

Human eosinophil derived neurotoxin:

5'-CCC CBB CBG BBG BBG CBG BCB BBT TTG GGB BGT
 GBB CBG TTT TGG BBC CBT GTT TCC TGT-3'

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des-adenosine antisense sequences:

HSEOSDNAS1: GCC CTG CTG CTC TTT CTG CT (SEQ ID NO:19)

5 HSEOSDNAS 2: TCC CTT GGT GGG TTG GGC C
 HSEOSDNAS 3: GCT GGT TGT TCT GGG GTT C
 HSEOSDNAS 4: TTG CTG CCC CTT CTG TCC C
 HSEOSDNAS 5: TGT TTG CTG GTG TCT GCG C

Human eosinophil major basic protein:

GGG GGB GTT TCB TCT TGG CTT T

10 *des-adenosine antisense sequences:*

TCT CCC CTT GTT CCT CCC C

TCT CCT GCT CTG GTG TCT CCT C

TTC CCT CCC TCC CCT GCC

GTG TTG TCT GTG GGT GTC C

15 GTT TCG CTC TTG TTG CCC

TGG GCC CTT CCC TGC TGG

Human eosinophil peroxidase:5'-GCB CCG TCC BGT GBT GGT GCG GTB CTT GTC GCT
GCB GCG CTC GGC CTG GTC CCG GBG BGC-3'20 *des-adenosine antisense sequences:*

HSEPAS1: GCGCTCGGCCTGGTCCCGG (SEQ ID NO:20)

HSEPAS2: GGG TCT CCT CTT GTT GTT GC

HSEPAS3: TTG CGC CTC CTG CTG GGG GT CC

HSEPAS4: CTC TGT TCT TGT TTT GGG GGC

25 HSEPAS5: GGG CCC GGC CGT TGT CTT G

HSEPAS6: GTT TGG GGG TTT CCG TTG

HSEPAS7: GGG TTC TCC TGG CCC GGG CCT TGC CC

HSEPAS8: GGC CGT GGT CCC GGC TTC GTT GC

HSEPAS9: CCT GTC TCC GTC TCG GCT CTT CTG

30 HSEPAS10: GGG CCT TGC GCT GTC TTT GGT G

Human intercellular adhesion molecule-1 (CAM-1):

35 5' - CGG BGC CTC CCC GGG GCB GGB TGB CTT TTG BGG
 GGG BCB CBG BTG TCT GGG CBT TGC CBG GTC CTG GGB
 BCB GBG CCC CGB GCB GGB CCB GGB GTG CGG GCB GCG
 CGG GCC GGG GGC TGC TGG GBG CCB TBG CGB GGC TGB
 G-3'

des-adenosine antisense sequences:

HSICAM1AS1: GCGCGGGCCGGGGGCTGCTGGG (SEQ ID NO:21)

40 HSICAM1AS2: GGT TGG CCC GGG GTG CCC C

HSICAM1AS3: GCC GCT GGG TGC CCT CGT CCTCTGCGGTC

HSICAM1AS4: GTG TCT CCT GGC TCT GGT TCC CC

45 HSICAM1AS5: 5'-GCT GCG CCC GTT GTC CTC TGG GGT
GGCCTTC-3'

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HSICAM1AS6: GCT CCC GGG TCT GGT TCT TGT GT
 HSICAM1AS7: TGG GGG TCC CTT TTT GGG CCT GTT GT
 HSICAM1AS8: GGC GTG GCT TGT GTG TTC GGT TTC
 HSICAM1AS9: TGC CCT GTC CTC CGG CGT CCC

5 Human vascular cell adhesion molecule 1 (VCAM-1):

5'-CTG BGC BBG BTB TCT BGB TTC TGG GGT GGT CTC
 GBT TTT BBBB GCT TGB GBB GCT GCB BBC BTT BTC
 CBB BGT BTB TTT GBG GCT CCB BGG BTC BCG BCC BTC
 TTC CCB GGC BTT TTB BGT TGC TGT CGT -3'

10 des-adenosine antisense sequences:

HSVCAM1AS1: CCTCTTTTCTGTTTTTCCC (SEQ ID NO:22)
 HSVCAM1AS2: CTC TGC CTT TGT TTG GGT TCG
 HSVCAM1AS3: CTT CCT TTC TGC TTC TTC C
 HSVCAM1AS4: CTGTGTCCTGTCTCCGCTTTTTTCTTC
 15 HSVCAM1AS5: GTC TTT GTT GTT TTC TCT TCC TTG

Human endothelial leukocyte adhesion molecule (ELAM-1):

5'-BBG TGB GBG CTG BGB GBB BCT GTG BBG CBB TCB
 TGB CTT CBB GBG TTC TTT TCB CCC -3'

des-adenosine antisense sequences:

20 HUMELAM1AAS1: GTTCTTGGCTTCTTCTGTC (SEQ ID NO:23)
 HUMELAM1AAS2: CGT TGG CTT CTC GTT GTC CC
 HUMELAM1AAS3: TGT GGG CTT CTC GTT GTC CC
 HUMELAM1AAS4: CCC TTC GGG GGC TGG TGG
 HUMELAM1AAS5: GGC CGT CCT TGC CTG CTG G

25 Human P Selectin:

des-adenosine antisense sequences:

HUMPSELECTAS1: CTCTGCTGGT TTTCTGCCTT CTGCCC
 (SEQ ID NO:24)

Human endothelial monocyte activating factor:

30 des-adenosine antisense sequences:

HUMEMAPIIAS1: 5'-TTT TCT CTT TCG CTT TCT TTT
 CGTCTCCTGTTCTCCTTTT-3' (SEQ ID
 NO:25)

35 HUMEMAPIIAS2: 5'-TTG CTG TTT TTT CTC CTT CTT
 CTC TCC TTT CTT TTC -3'

Human IL3:

5'-GGCGGBCCBGGBGTTGGBGCBGGBGCBBGCGGGCB
 GGCGGCTCBTGTGTTGGBTCGGCBGGBGGCBCTC -3'

des-adenosine antisense sequences:

40 HUMIL3AAS1: 5'-CTC TGT CTT GTT CTG GTC CTT CGT
 GGG GCT CTG (SEQ ID NO:26)-3'
 HUMIL3AAS2: TGT CGC GTG G GTG CGG CCG TGG CC

Human IL3 receptor:

45 5'-GCBGGBGBCBGGGCBGGGCBTCBGGBGCBCGCT
 GBGCCBBBGGBGGBCBCTCGGGBBCGCBGCTCCG
 GBBCGCBGGBCBGGGTGCC-3'

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des-adenosine antisense sequences:
TCTGGGGTGTCTG

GCCTTCGTGGTTCC

5 TCTTCCTTCGTTTGC

CGTCCGCGGGGGCCCCCGGGCCT

GGCTGCGCTCCTGCCCCGC

CTCTTTCCCGGGCTCTT

10 GCGCTGGGGGGTGCTCC

CGTGTGTTTGCGCCCTCCTCCTGGTCGC

GCTTGTGCTTTTGG

15 GGCCGGCTTTGCCCGCCTCCC

GGCGCCTGGCCCCGGCC

TTCCTGGGCTGCGTGCGC

20 GTTCTGTTCTTCTTCCTGGC

Human IL4:

5' -GCCGGCBGCTGCTBGCBGGBBGBBCBGBGGGGGB
BGCBBGTTGGGBGGTGBGBCCCBTTBBTBGGTGTGCB-3'

25

des-adenosine antisense sequences:
HUMIL4AS1: CTC TGG TTG GCT TCC TTC-3'
(SEQ ID NO:27)

Human IL4 receptor:

30 5' -GTTCCCBGBGCTTGCCBCCTGCBGCBGGBCCBGGCBGCTC
BCBGGGBBGBGGGCCCCBGBGCBBBGCCBCCCCBTTGGGBG
BTGCCBBGGCBCCBGGCTG-3'

des-adenosine antisense sequences:
TCTGCGCGCCCTGCTCC

35

CGCCCCGGCTTCTCT

CGTGTGGGCTTCGG

40

CCCCGCGCCTCCGTTGTTCTC

TGCTCGCTGGGCTTG

GGTTTCCTGGGGCCCTGGGTTTC

45

TCTGCCGGGTCGTTTTC

GGGTGCTGGCTGCG

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CTTGGTGCTGGGGCTCC
GGCGGCTGCGGGCTGGGTTGGG
5 CTTGGCTGGTTCCTGGCCTCGGG
CCTCCTCCTCCTCCTC
10 GCTCCCTTTTCTTCCTCT
TCCCTGCTGCTCTC
TGCCCTCCCTTCCCTCCTGG
15 GGTGCCTCCTTGGGCCCTGC
GGCTGCTCCTTGCCCC
20 CTCTGGGTGCGGGCTGGC
GGGGCGTCTCTGTGC
CTGGCCTGGGTGCC
25 GCCTCTCCTGGGGG
GGTGGCTCCCTGTCC
CCTTTTCCCCCGGCTCC
30 GTGGGGGCTTTGGC
GGGGGTCTGTGGCCTGCTCCTGGGG
35 AGGGGTCTGGGGCCCTC
TTTGGGGGTCTGGCTTG
GCCTGGCTGCCTTCC
40 GGGGCCTGCCGTGGGGC
TGTCTCTGTTGCTCCCCTT
45 TGCCTGCTGTCTGG
GGTTCCCGCCTTCCCT

Human IL5:

50 5' -GTGGGBBTTTCTGTGGGGBTGGCBTBCBCGTBGGCB
GCTCCBBGBGCTBGCBBCCTCBBBTGCBGBBGCBTC
CTCBTGGCTCTGBBBCG -3'

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des-adenosine antisense sequences:

HUMIL5AS1: TCC CTG TTT CCC CCC TTT (SEQ ID
NO:28)
HUMIL5AS2: CGT TCT GCG TTT GCC TTT GGC
5 HUMIL5AS3: GTT TTT TGT TTG TTT TCT
HUMIL5AS4: CTC TCC GTC TTT CTT CTC C
HUMIL5AS5: CCT CCT GCC TGT GTC CCT GCT CCC C
HUMIL5AS6: GAG GGT TTC TGG CTT CCT CTC T
HUMIL5AS7: TGT CTC TCT GTC CTT TTG TT
10 HUMIL5AS8: 5'-TGT TGT GCG GCC TGG TGC TGC CCT
GCCCG GG-3'

Human IL5 receptor antisense oligonucleotide

5'-CTCBGTGGCCCCBBBGGBT
GBGTBBTBCBTGCGCCBCGBT
15 GBTCBTBTCCTTTTBTBTBGG-3'

des-adenosine antisense sequences:

CCGTGTCTGTCGTGTCT
TTCCTTTGCTCTTG
20 GTGTGTCTTTGCTGT
GCCCTGCCTCTCTGC

25 **Human IL6:**

5'-CTCCTGGGGTBCTGGGGCBGGGBB
GGCBGCBGGCBBCBCCBGGBGCBCG
CCCBGGGBGBBGGCBCTGGBCCGB
BGGCGCTTGTGGBBGGBTTCBT
30 BGCTGGGCTCCTGGBGGGBGBTBGBGC-3'

des-adenosine antisense sequence:

HUMIL6AS1: GCT TCT CTT TCG TTC CCG GTG GGC TCG
(SEQ ID NO:29)
HUMIL6AS2: GTG GCT GTC TGT GTG GGG CGG CT
35 HUMIL6AS3: GTG CCT CTT TGC TGC TTT C
HUMIL6AS4: GAT TCT TTG CCT TTT TCT GC

Human IL6 receptor antisense oligonucleotides

5'-GCBGCTCTTGCCBCCTCCTGCGCBGGGCB
40 GCGCCTTGGGGCCBGCCTGCTCCCGGCGCG
GCCBGCBBGGCBGCBGCGCGCBGCCGB
CGGCCBGCBTGCTTCCTCCTCGGCTBCCBCT
CCBTGGTCCCGCBGBGGCGGBCBGGC-3'

des-adenosine antisense sequences:

GGGGGTGGCTTCCTGCC
45 GCGTCTCTGGGCCGTCCC
GTCCCTCGGCCCCGCGCGCTCGGCTCCTCTCCC
TCTGGCCCGGCTC

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GGGGCGGGGCGGGGCGGTGGGCGGGC
GGCGCTGCCCTGCGC
5 GCGGCGCTGGCCCC
TGCTGGCCGTCGGCTGCGCGCTGCTGGCTGCCCT
GCTGGCCGCGCCGGG
10 GCCTGTCCGCCTCTGCGGG
CGCTGTCTCCTGGC
TTGTCTTCCGGCTCT
TCTGCTGGGGTGGG
15 GCTGGGCGGCCGGCCCGGT
GCTGGGGCTCCTCGGGGGG
20 GGGGGCTCTTCCGG
GCTGTCTCCCTCCGGG
GCGGGGGTTTCTGGCC
25 GTGGGGGTCTTGCC
TGGCCTCCGGGCTCC
30 TGCTTGTCTTGCCTTCCTTC
TCTGGTCGGTTGTGGCTCG
GGGCTCCGTGGGTCCCTGGC
35 GCCCGTTTGTGTTTTGTC
TTTTCCCCTGGCGT
40 CCTGTGCCCCCTCTCCTCTCCTTCCTCTGCTTCTC
GCTCTCCTTTGTGGG
GCCCTCCCTGCTGCT
45 CTTGGTTTTTGGGCT
TTTTTTCTCTTCCTCCTTTTTC
50 GTGCGTGGGCCTCC

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Human monocyte-derived neutrophil chemotactic factor:

5'-GGGGTGGBBBGGTTTGGBTBTGTCTTTBTGCBCTGB
 CBTCTBBGTTCTTTBGCBCCTCTGGCBBBBCTGCBC
 CTTCBBCBGBGCTGCBGBBBTCBGGBBGGCTGCCBB
 5 GBGBGCCBCGGCCBGCTTGGBBGTCBTGTTTBCBCBC
 BGTGBGBTGGTTCCTTCCGG-3'

des-adenosine antisense sequences:

HSMDNCFAS1: GCT TGT GTG CTC TGC TGT CTC T (SEQ
 ID NO:30)
 10 HSMDNCFAS2: 5'-TGG TTC CTT CCG GTG GTT TCT TCC
 TGG CTC TTG TCC T -3'
 HSMDNCFAS3: TTC TCT TGG CCC TTG GC

Human neutrophil elastase (medullasin):

5'-GGGCTCCCGCCGCGBGBGGTTBTGGGCTCCCBGGBCCBC
 15 CCGCBCCGCGCGGBCGTTTBCBTTCCGCCBCGCBGTGCGC
 GGCCGBCBTGBCBGGTTGGGCGCBBTBGGGTGGCGCC
 GCBGBBGTGGCTCCGCGCBGCTGCBGGGBCBCCBTGBB
 GGGCCBCGCGTGGGGCCGCGCTCGCCGGCCCCCBBB
 CTCCGCGGCGCGCGGTGCCCCCBBGCBGCBGGCCGG
 20 CBGGBCBGBGGCGBGGBCBGBGCGCGGTGCGCGGCCGCG
 GGTCTGGTGGGGCTGGGGCTCCGGGGTCTCTGCCCTC
 CGTGC-3'

des-adenosine antisense oligonucleotides:

HSMEDURAS1: 5'-TGG TGG GGC TGG GGC TCC GGG GTC
 25 TCT GCC CCT CCG TGC-3' (SEQ ID NO:31)
 HSMEDURAS2: CGC GTG GGG CCG CGC TCG CCG GCCCCC
 HSMEDURAS3: CCT GCC GGG TGG GCT CCC GCC GCG
 HSMEDURAS4: CGC CGG CCT GCC GGC CCC TC
 HSMEDURAS5: 5'-GTG GGT CCT GCT GGC CGG GTC CGG
 30 GTC CCG GGG GTG GGG-3'
 HSMEDURAS6: CGC GBG TCG GCG GCC GBG GGT C

Human neutrophil oxidase factor:

5'-CGGGBGTGGGGGTCTTGGBCGGCBCTGBBGGCBTCCBGGG
 35 CTCCCTTCCBGTCTTCTTGTCCGCTGCCBGCBCCTTC
 BTCCBGBGGCTGCTGGCCTCCBCCBGGGBCBTGTTBGG
 TBGBBCTBGGGGGCC-3'

des-adenosine antisense sequence:

HUMNOXFAS1: GGC CTC CBC CBG GGB CBT G (SEQ ID
 40 NO:32)
 HUMNOXFAS2: GTC CTT CTT GTC CGC TGC C
 HUMNOXFAS3: TCT CTG GGG TTT TCG GTC TGG GTG G
 HUMNOXFAS4: GCT TTC CTC CTG GGG CTG CTG CTG
 HUMNOXFAS5: 5'-GGC TCT TCT TTT TGT TTC TGG CCT
 45 GGTG-3'
 HUMNOXFAS6: CTC TCT CGT GCC CTT TCC
 HUMNOXFAS7: CTT GGG TGT CTT GTT TTT GT
 HUMNOXFAS8: 5'-GGCCTCCBCCBGGGBCBTGGTCTTCTT
 GTCCGCTGCC -3'

Human cathepsin G:

5' -CCCTCCBCBTCTGCTCTGBCCTGCTGGBCTCTG
 GBTCTGBBGBTBCGCCBTGTBGGGGCGGGBGTG
 GGGCCTGCTCTCCCGGCCTCCGBTGBTCTCCCT
 5 GCCTCBGCCCCBTGGGTBGGGBBBGGCCBGC
 GBBGCBGGBTGGCTGCBTCTTTCCTG -3'

des-adenosine antisense sequences:

HUMCTHGAS1: GTG GGG CCT GCT CTC CCG GCC TCC G
 (SEQ ID NO:33)
 10 HUMCTHGAS2: TGTGTTGCTGGGTGTTTTCCCGTCTCTGG
 HUMCTHGAS3: TCT GCC TTC GGG GGT CGT

Human defensin 1:

5' -CCGGGGCTGCBGCBBCCTCBTCBGCTCTTGCCT
 GGBGTGGCTCBGCCTGGGCCTGCBGGGCCBCCB
 15 GGBGBBTGGCBGCBGGBTGGCGBGGGTCTCTCB
 TGGCTGGGGTTCBGBTCCTCTBGCTBGGCBGG
 GTGBCCBGBGBGGGC-3'

des-adenosine antisense sequences:

HUMDEF1AAAS1: GGG TCC TCB TGG CTG GGG (SEQ ID
 20 NO:34)
 HUMDEF1AAAS2: GCC TGG GCC TGC BGG GCC
 HUMDEF1AAAS3: GCT CTT GCC TGG BGT GGC TC
 HUMDEF1AAAS4: GCC CBG BGT CTT CCC TGG T

Human defensin 3:

5' -CGCTGCBBTCTGCTCCGGGGCTGCBGCBBCCTCBTC
 25 BGCTCTTGCTGGGTGGCTCBGCCTGGGCCTGCBG
 GGCCBCCBGGGBBTGGCBGCBGGBTGGCGBGGGT
 CCTCBTGGCTGGGGTCCCTGGBGGGBGGGBGCBGG-3'

des-adenosine antisense sequences:

HUMNTRIIIAS1: GGG TCC TCB TGG CTG GGG TC (SEQ
 30 ID NO:35)
 HUMNTRIIIAS2: CCT CTC TCC CGT CCT

Human macrophage inflammatory protein-1-alpha: RANTES RECEPTOR

5' -GBGGGGGCBGCBGTTGGGCCCCBBBGGCCCTCTCGT
 35 TCBCTTCTGGCBGCGGTTGCBTCCCCBTBGTCTBB
 BCTCTGTGGTCTGTCTBGTCTCTGTGGTGTGTTG
 GBGTTTCCBTCCCGGCTTCTCTCTGTTCCBBGGGB-3'

des-adenosine antisense sequences:

HUMRANTESAS1: GTC TTT GTT TCT GGG CTC GTG CC
 (SEQ ID NO:36)
 40 HUMRANTESAS2: CCB TCC CGG CTT CTC TCT GGT TCC
 HUMRANTESAS3: GTC CTCTGT GGT GTT TGG
 HUMRANTESAS4: 5' -CCC TGC TTC CTT TTG CCT GTT
 45 TCTTTGTTT CTGGGCTCGT GCC -3'

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RANTES:

5' -GGGCBGCGGGGCBGTGGGCGGGCBBTGTBGGC
 BBBGCBGCBGGGTGTGGTGTCCGBGGBBTBTGGG
 GBGGCBGBTGCBGGGCGCBGBGGGCBGTBGCBB
 TGBGGBTGBCBGGCGGGCGTCCGCGGBGBCCTTC
 BTGGTBCCTGTGGGBGGCTGTCGGBGG-3'

des-adenosine antisense sequences:

GGGTGTGGTGTCCG
 CTTGGCGGTTCTTTTCGGGTG
 TTTCTTCTCTGGGTGGC
 CTGCTGCTCGTCGTGGTC
 GCTCCGCTCCCGGGTTC
 GTCTCGCTCTGTGCCCC
 CTCCTTCCTTGTC
 GTGTTCCCTCCCTTCCTGCCTCT

Human muscarinic acetylcholine receptor HM1:

des-adenosine antisense sequences:
 HSHM1AS1: GTT CBT GGT GGC TBG GTG GGG C (SEQ ID NO:37)
 HSHM1AS2: GCT GCC CGG CGG GGT GTG CGC TTG GC
 HSHM1AS3: GCTCCCGTG CTC GGT TCT CTG TCTCCCGGT
 HSHM1AS4: CCC CCT TTG CCT GGC GTC TCG G
 HSHM1AS5: GCC TTC GTC CTC TTC CTC TTC TTC CTTCC
 HSHM1AS6: 5'-GCT CCG TGG GGG CTG CTTGGTGGG
 GGCCTG TGC CTC GGG GTC C-3'
 HSHM1AS7: CGG GGC TTC TGG CCC TTG CC

35 Human muscarinic acetylcholine receptor HM3:

des-adenosine antisense sequences:
 HSHM3AS1: GGG GTG GGT BGG CCG TGT CTG GGG (SEQ ID NO:38)
 HSHM3AS2: GTT GGC CBT GTT GGT TGC C
 HSHM3AS3: TCT TGG TGG TGC GCC GGG C
 HSHM3AS4: 5'-GCG TCT TGG CTT TCT TCT CCT TCG
 GGC CCT CGG GCC GGT GCT TGT GG-3'
 HSHM3AS5: 5'-GCT CCT CCC GGG CGG CCT CCC CGG
 GCG GGG GCT TCT TG-3'
 HSHM3AS6: GCG CTG GCG GGG GGG CCT CCT CC
 HSHM3AS7: 5'-GCT CTG TGG CTG GGC GTT CCT TGG
 TGT TCT GGG TGG C-3'
 HSHM3AS8: TGG CGG GCG TGG TGG CCT CTG TGG TGG
 HSHM3AS9: GGG CCC GCG GCT GCB GGG G
 HSHM3AS10: TTG CCT GTC TGC TTC GTC
 HSHM3AS11: CTT TGC GCT CCC GGG CCG CC

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Human fibronectin:*des-adenosine antisense sequences:*

HUMFNA/HSFIB1AS1: CGG TTT CCT TTG CGG TC (SEQ ID NO:39)

5 HUMFNA/HSFIB1AS2: TTG GCC CGG GCT CCG GGT G
 HUMFNA/HSFIB1AS3: CCC GCC CGC CCG CCG GCC GCCGC
 HUMFNA/HSFIB1AS4: 5'-CCC GCC GGG CTG TCC CCG
 CCC CGC CCC-3'

10 HUMFNA/HSFIB1AS5: GGC CCG GGG CGC GGG GG
 HUMFNA/HSFIB1AS6: CGG CCC TCC CGC CCC TCT GG
 HUMFNA/HSFIB1AS7: GCC GGC GCG GGC GTC GG
 HUMFNA/HSFIB1AS9: 5'-CCG CTC GCG CCT GGG GTT
 CCC TCT CCT CCCCCTGTGC-3'

15 HUMFNA/HSFIB1AS10: GCC TGC CTC TTG CTC TTC
 HUMFNA/HSFIB1AS11: TGC GTC CGC TGC CTT CTC CC
 HUMFNA/HSFIB1AS12: CTC TCC TCG GCC GTT GCCTGTGC
 HUMFNA/HSFIB1AS13: 5'-TGT CCG TCC TGT CGC CCT
 TCC GTG GTG C-3'

20 HUMFNA/HSFIB1AS14: TGT TGT CTC TTC TGC CCT C
 HUMFNA/HSFIB1AS15: GGT GTG CTG GTG CTGGTGGTGGTG
 HUMFNA/HSFIB1AS16: CCT CTG CCC GTG CTC GCC
 HUMFNA/HSFIB1AS17: CTG CCT GGG CTG GCCTCTTCGGGT
 HUMFNA/HSFIB1AS18: 5'-GTG GCT TTG GGG CTC TCT
 TGG TTG CCC TTT-3'

25 HUMFNA/HSFIB1AS19: 5'-CTT CTC GTG GTG CCT CTC
 CTC CCT GGC TTG GTC GT-3'

HUMFNA/HSFIB1AS20: TGT CTG GGG TGG TGCTCCTCTCCC
 HUMFNA/HSFIB1AS21: TTT CCC TGC TGG CCG TTT GT
 HUMFNA/HSFIB1AS22: CCT GTT TTC TGT CTT CCT CT
 30 HUMFNA/HSFIB1AS23: TTC CTC CTG TTT CTC CGT
 HUMFNA/HSFIB1AS24: 5'-TTG GCT TGC TGC TTG CGG
 GGC TGT CTC C-3'

HUMFNA/HSFIB1AS25: CTT GCC CCT GTG GGC TTT CCC
 HUMFNA/HSFIB1AS26: TGG TCC GGT CTTCTCCTTGGGGGTC
 35 HUMFNA/HSFIB1AS27: GCC CTT CTT GGT GGG CTG
 HUMFNA/HSFIB1AS28: GCT CGT CTG TCT TTT TCC TTCC
 HUMFNA/HSFIB1AS29: 5'-TGG GGG TGG CCG TTG TGG
 GCG GTG TGG TCC GCC T-3'

HUMFNA/HSFIB1AS30: TGC CTC TGC TGG TCT TTC

40 Human interleukin 8:

5'-GBTGTTTGTBCCBBBGCBCBGBBGTBGCTTTGC
 TBTCTBBGGBTCBCBTTTBGBCBTBGGBBBBBCGC
 TGTEGGTCBGBBBGBTGTGCTTBCCTTCBCBCBG
 BGCTGCBGBBBTBCGGBGGCTGCCBBGBGBGCC
 45 BCGGCCBGCTTGGBGTCTGTGTTTBCBCBCBGTGBG-3'

des-adenosine antisense sequences:

HUMIL8AAS1: GTG CTC CGG TGG CTT TTT (SEQ ID NO:40)

50 HUMIL8AAS2: GCT TGT GTG CTC TGC TGT CTC TG
 HUMIL8AAS3: 5'-TTC CTT CCG GTG GTT TCT TCC TGG
 CTC TTG TCC T-3'

HUMIL8AAS4: TTC TCT TGG CCC TTG GCC C

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Human IL-8 receptor-alpha

5' - BCBGGGGCTGTBBTCTTCBTCTGCBGGTGGCB
 TGCCBGTGBBBTTTBGBTCBTCBBBBTCCBCBT
 CTGTGGBTCTGTBBTBTBTGBCBTGTCCTCTTC
 5 BGTTCBGCBBTGGTTTGBTCTBBCTGBBGCBCCG
 GCCBGG-3'

des-adenosine antisense sequences:
 TGGCTCGGTGCTTCTGCCCC

TGTTGTTGCGGCGCTC
 10 GGTGGTGTGGCCCCTG
 TGGTGCTTCGTTTCC
 15 CCCTCTTCTCTTTGTTT
 GGGGGTTCTTGTGGC
 GGGCTGCTTGTCTCGTTCC

20 Human GM-CSF:

5' - CTTGBGCBGGBGCTCTGGGGCBGGGBGCTGGCBG
 GGCCCBGGGGGGTGGCTTCTGCBCTGTCCBGBGT
 GCBCTGTGCCBCBGCBCGCTGCBGGGGCCBCTCBG
 25 CTTCBTGGGGCTCTGGGTGGCBGGTCCBGGCCBTGG
 GTCTGGGTGGGGCTGGGCTGCBGGCTCCGGGC-3'

des-adenosine antisense sequences:
 HUMGCSFAS1: GGT CCB GCC BTG GGT CTG GG (SEQ ID
 NO:41)
 HUMGCSFAS2: GGC TGG GCT GCB GGC TCC GG
 30 HUMGCSFAS3: GCG GGC GGG TGC GGG CTG CGT GCT GGG
 HUMGCSFAS4: GGC TGC CCC GCA GGC CCT GC

Human tumor necrosis factor α:

5' - CBCCGCCTGGBGCCCTGGGGCCCCCTGTCTTCTTGGG
 GBGCGCCTCCTCGGCCBGTCCBCGTCCCGGBTCBTGCTTT
 35 CBGTGCTCBTGGTGTCTTTCCBGGGGBGBBGGG-3'

des-adenosine antisense sequences
 HSTNFAAS1: GCT GGT CCT CTG CTG TCC TTG CTG (SEQ
 ID NO:42)
 HSTNFAAS2: GTG CTC BTG GTG TCC TTT CC
 40 HSTNFAAS3: GCC CTG GGG CCC CCC TGT CTT CTT GGG
 HSTNFAAS4: CCT CTT CCC TCT GGG GGC CG
 HSTNFAAS5: TCT CTC TCC CTC TCT TGC GTC TCT C
 HSTNFAAS6: TCT TTC TCT CTC TCT CTT CCC C
 HSTNFAAS7: TTT CCC GCT CTT TCT GTC TC
 45 HSTNFAAS8: GGT GTC TGG TTT TCT CTC TCC
 HSTNFAAS9: GCT GGC TGC CTG TCT GGC CTG CGC TCTT
 HSTNFAAS10: GGC CTG TGC TGT TCC TCC
 HSTNFAAS11: TCC GGT TCC TGT CCT CTC TGT CTG TC
 HSTNFAAS12: GCC CCC TCT GGG GTC TCC CTC TGG C
 50 HSTNFAAS13: GTG GTG GTC TTG TTG CTT

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HSTNFAAS14: GGG CTG GGC TCC GTG TCT C
 HSTNFAAS15: CBG TGC TCB TGG TGT CC
 HSTNFAAS16: GCT GBG GGB GCG TCT GCT GGC

Human leukotriene C4 synthase:

5 5'-CTCGGTBGBCGCGCTCGBBCTCGGGTGGGCCGGTGGTG
 BGCGGCGGCGBCBCGCGGBGGCCCTGCGCGCCGBGBCBC
 CTGCBGGGBGGBBGTBGGCTTGCBGCBGGBTCCCBGGGBGGG
 TGBCBGCBGCCBGTBGBGCTBCCTCGTCCTTCBTGGTBCCG
 TCGGTGTGGTGGCBGCGGGCTGTGTGTGBBGGCGBGCTGG-3'

10 *des-adenosine antisense sequences:*
 HSU11552AS1: GCC CCG TCT GCT GCT CCT CGT GCC G
 (SEQ ID NO:43)
 HSU11552AS2: 5'-CCT CGT CCT TCA TGG TAC CGT
 CGGTGT GGT GGC-3'

15 HSU11552AS3: CTC GGG TGG GCC GGT GGT G
 HSU11552AS4: GGG CGC GCG CGC TCG CGT
 HSU11552AS5: 5'-GGC TCC GGC TCT TCT TTC CCG
 GCTCCG TCG GCC CGG GGG CCTTGGTCTC-3'
 HSU11551AS6: CCT CGT CCT TCB TGG TBC CG

Human Endothelin-1:

20 5'-BCCGGCGGBGCCGCCBGGGTGGBCTGGGBGTGGGTT
 TCTCCCCGCCGTTCTCBCCCBCCGCGCTGBGCTCBGCGC
 CTBBGBCTGCTGTTTCTGGBGCTCCTTGGCBGCCBCBB
 BCBGCBGBGBBBBTCTBTBGCBBBTBBTCCBTTCTGB
 25 BBBBBBGGGBTB BBBBCCTCCCGT-3'

des-adenosine antisense sequences:
 CCCGTTGCGCTGGCGC
 GCGCTGCGGGTTCCTC
 GTGGGTTTCTCCCCGCCGTTCTC
 30 CGGTCTGTTGCCTTTGTGGG
 CTTCTTGTCTTTTTGGCT
 GTTCTTTTCCTGCTTGGC
 GTCTTTTCCTTTCTT
 TGTGCTCGGTTGTGGGTC
 35 CGCTGGTCCTTTGCC
 CTGTGTGTTTCTGCTG

Endothelin receptor ET-B antisense oligonucleotides

40 5'-GCCCTGTCGGGCGGGBGGCCTCTCTCCTCTCCCCBG
 BTCCGCGBCBGGCCGCBGGCBGBBCCBGCGBBCCBGG
 GCGCGTCCGCBGBCTTGGBGGCGGCTGCBTGCTGCTB
 CCTGCTCCBGBBGGCGTCCGGTGGCCGCCGC-3'

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des-adenosine antisense sequences:
GCGTCCGGTGGCCGCCGC

GCCTCTCTCCTCTCCCC

GTGGCCCTGTGCGGCGGG

5 TCCTGCCGTCTGTCTCCTTT

TCTTTTGCTGTCTTGT

CTTCCCGTCTCTGCTTT

Endothelin ETA receptor antisense oligonucleotides

10 5' -CBTCCBCBTGBTTGCTTBGBTTTGTGCTGTBTCTCTCB
GGBTTBTCTGBTTBCBCBTCCBBCCBGTCGCCGCCBBBB
GGBTGCCCTGBGGCBBBGGGTTTCCBTCTTGBGGCBBBTTT
GBGGB-3'

des-adenosine antisense sequences:
GTCTGTCTCTCCCCGTCTCCTCCC

15 ACTGCTTCTCCCGGGG

GCTTCCCCGGCTTC

GGGTGGCCGGTGTCCCGGGCTCCGGCGCGGCGGC

20 GGCTTCGGCTGC

GGGTGGGTGGCGCGG

GCTGCCGGGTCCGCGCGGCGCCTGGGCC

25 CTTGTGCTGCTTTT

TGCTTGTTCCGTTC

TGGCTGCTCCGGTCTGTGTTGTGGTTGTTTTG

TTTCTTCTTGGGTGTGGG

30 CCTTGCGGTTTTGG

CTGTGGGCCCTTTG

35 GGGCCTTGGCTTCTGGCTC

Substance P antisense oligonucleotide

40 5' -CTGCTGBGGCTTGGGTCTCCGGGCGBTCTCTGCBGBBGBT
GCTCBBBGGGCTCCGGCBGTTCTCCTTGBTCTGGTCGCTGTCTG
TBCCBGTCGGBCCBGTBBTTCBGBTCBTCTTGGCTCCTBTTC
TTCTGCBBCBGCTGBGTGGBBCBBBGBBGBCTGCCBBGG
CCBCGBGGBTTCBTGTTGGBTTTTGCGBCGGBCBGTCCCGCG
GGGTGCTGAGTTTCTCTGGTTCTCCGBGCGCB-3'

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des-adenosine antisense sequences:
CGTGGTCGCTCCGC

TTTCTCTGGTTCCTCCG

GTCCCGCGGGGTGCTG

5 TCTGGTCGCTGTCGT

GGCTTGGGTCTCCGGGCG

GTTTCCTTCCTTTTCCGC

10 **Substance P receptor antisense oligonucleotide**
5'-GGCTBBGBTGBTCBCBTCBCTBCCBCGTTGCCCCBCCBCB
GBGGTCBCCBCBBTGBCCGTGTBGGCBGCTGCCBBBGGBCBB
TTTGCCBGGCTGGTTGCBGBBCTGBTTGGGTTCGBGGTGTT
BGTGGGBTGTTTGGGGBGBGGTCTGBGTCCBCCGGGBGGBCG
TTBTCCBTTCGBBGCTBGGCGGTBBBGCCCTBCTBTCTGTBC
15 BCBCCCCCTCTGCBGCBGBGTCTGTCTGTGGCGCCTGGGGC
TCBGGGTCC-3'

des-adenosine antisense sequences:
GTCCTGTCGTGGCGCCTGGGGCTC

20 TTCTTTTGTGGGCT

CTTTGGTGGCTGTGGCTG

TGGTCTCTGTGGTTG

25 CTGCCCTGGGTCTGG

GGGTGTGGCCTTGGGGCCGTCTCTGGCTCCTCCTCGTGGGCCCCC

Chymase
30 5'-GGBGCTGBTBCTGCBGATTTCBGBGGGBBGBBCCCT
GBTBCTCBCCBGCTTCBGCTCTGGBGCBGBBGBBBGB
GCBGCBGGGGGBGBBGBBGBBGBBGBBGBBGBBGBBGBB
GGCTGCCTGBGCBBBTGCTGGTTTTCTTTCCBGTCTTG
GGTTTTBTBBCTCCCBGBBGGCBGBBGGGGCBGG-3'

des-adenosine antisense sequences:
35 CGTTTTCTTCTCTC

TGCTGGTTTTCTTTCC

TGGCAGTGGGTGGGGGTGGGGGTGGGGTGGC

40 TTCCTTGTTCTCTGGGGGTGTCCT

CTTGCTCTGGGCTTTTCT

45 CCCCTTTTCCTTCC

TGTCTGTTTTCTGGGG

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CTCTCCTCTGTCTCTGTGT
CCTTGCCCTGGCCC
5 TCTTCCCTCTCCTGTCTCCTGT
CCCTGTGTTCGCCC
GTCTTCCCTCTCCTG
10 ACCTCCTTTTCCTCCG
CTGGGTGGGGCCCTG
CCTGTTCTCTGCTCCC
TGGCTTGGGGTTTCTTCTG
15 TGTGTCTTCTTCCTCTGTT
GGCTGGCTTTCTCCTTC
TTTGTCTTCCTGGG
TGCCCTTCTTCCTTTCTTGGG
20 TCCTTGGTGCTTGGGCTGGG

Endothelial nitric oxide synthase

5' -GCGTCTTGGGGTGCBGGGCCCBTCCTGCTGCGCCTGGGCG
CTGBGGGTGTCBTBGGTGBTGCTCCCCBCCTCCCBGTTCTTCB
CBCGBGGGBBCTTGGGCCCCCTCTGGGGGCTGGGTTBGCGGGGB
25 GCTCGGGGGGCTGTGTTCTGGCGCTGGTGGGBGTBGGGBTGCT
GGGGCCCGGCTGGGCTCBGGGGCCGGGGTGGCTGGGCCCTGCT
TGCCGCBGCCCCBBGGCCCBGCCCCBGCCCCBGCCGCBGGG
TGGCCCBGGCTCCTGGGCCBCGCTCTTCBBGTTGCCCBTGTB
CTGTGCGTCCGTCTGCTGGBGCBGGCBGCBGTTGGGBBTTTC-3'

30 *des-adenosine antisense sequences:*
CTGTGCGTCCGTCTGCTGG

GGGGCCGGGGTGGCTGGGCCCTGCTTGCCGC
ACGACCCCGGGCCGACCCGAG
35 GCTCGGGGGGCTGTGTTCTGGCGCTGGTGGG
CTTGGGCCCCCTCTGGGGGCTGGGTT
TCCTGCTGCGCCTGGGCGCTG
GCGTCTTGGGGTGC
GGGGCCGGGGGGCCGGGGG
40 GCCGCTGTTCTGTGGGCCTGGG

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GGTGCCTGTGGCTGCC
GGTTGCCCCGGTTGGTGGC
GCCGTCCTGCTGCCGGT
CGTTGGCTGGGTCCCCCGC
5 CCGTTTCCTGGGGTCC
GCGTGGGGTGCTCC
GGTTCCTCGTGCCG
CTGCTGCCTTGTCTTTCC
GGCCGTGGCGGCGTGGTGGTCC
10 GCCCCCCTGGCCTTCTGCTC
GGGGTCTGGCTGGT
TGCCGGTGCCCTTGGCGGC
GGTCTTCTTCCTGGTG
GCTCTGGGCCCCGGCCGGTCTCGG
15 GCGTCTCGTGTTTCG
CTCTTGTGCTGTTCCGGCCG
CTCCTTCCTCTTCCGCCGCC
GCCGCTCCCCGCCC
20 GCTCGTCGCCCTGGCCC
GGCCTCCTCCTGGCCGC
TGTCTCGGGCGGCGGCCTTGGC
GCTCCGTTTGGGGCTG
CCTCTGGCGCTTCC
25 GGCCCTCGGCCTGGGCGCTC
TCTTCCGCCTGTGC
TGGTGGCCCTCGTGG
GCCCCCTCCTGGCCTCCGGTGTCC
TGTGGTCCCCCGGCTGGT

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GGCCGGGCGCGTTGGGCGGGC
 GTGGGCGCCGGCGGGTCCTCC
 GGGCTGCCCTTCTCC
 GCCGGGGGTCCCGC
 5 GCTCCTGCTGTTCCCTGGGCTCTTCTGCC
 TCTCTCCTGGGTGGGTGCTGGGTGCCG
 10 GGGTCTCCGGGCTTG
 CCCC GCGCTGCTGGGCGTTCTGC
 GGTCTTGGGGTTGTC
 15 TGTGGCCCCGCTCG
 TGTCGCCCTCCGTCGCC
 20 CGTCGCCGGCCTCGTCC
 CCTCCTGGGTGCGC
 GGC GGGCTGGTCCT
 25 GGC GTTTGTCTCCTTCCTGG
Inducible nitric oxide synthase
 5'-CTGCCCCBGT TTTTGBTCCTCBCBTGCCGTGGGGBGGB
 CBBTGGGGTTGCBTCCBGCTTGBCCBGBBTTCTGGBG
 30 BCTTCTTTCCCGTCTCCBCBGGGGCTGCGGGGBCTCB
 TTCTGCTGCTTGCTGBGGTTGTGBTBCTGBGGTCBTCC
 TGTGTCBCTGGBCTGGBGGTGGCBGBGGGGGCTTCTC
 CBCBTTGTTGTTGTTGTCTTTTTCCCCBTTCBTTGCBT
 BCTGGTGGBBTTTGGTCTTGBBGBBBTTTCCBBGGB
 35 CBGGCCBTCTCTBTGGCTTTBCBBBGBGGTCBCTTBT
 GTCBCTTBTCTGGBTTTGBGCTCBGBTGTTCTTCBCTG
 TGGGGCTTGCBGCTGGCTGCBCTGCCTCCCCGGGGTB-3'

Human major basic protein:
 GTTTCATCTT GGCTTTATCC (SEQ ID NO:44)

40 EXAMPLE 6

Turning now to Figure 3, two asthmatic rabbits
 were administered adenosine, and two rabbits were
 administered dAMP, at the indicated concentrations, by
 inhalation as described above in Example 3. The results
 45 (shown in Figure 3 as change in compliance) indicate
 that dAMP, a breakdown product of antisense

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oligodeoxynucleotides containing adenosine, is as potent in the induction of bronchoconstriction as adenosine in the hyperresponsive airways of asthmatic rabbits.

EXAMPLE 7

5 An aerosolized phosphorothioate 21-mer antisense ODN consisting of 50% adenosine and 50% guanine plus cytosine in a random configuration was found to produce potent bronchoconstrictor effects in hyperreactive airways of asthmatic rabbits, as
10 illustrated in Figure 4. The control molecule used in this study, a phosphorothioate 21-mer antisense ODN consisting of 50% guanine and 50% thymidine plus cytosine (*des*-adenosine ODN) produced no bronchoconstrictor or any other effect in these same animals.

15 In this study, bronchoconstrictor effects were measured as a percentage change in bronchial compliance. Each group consisted of two allergic rabbits, and data shown are for the period following the second of two daily administrations of 5 mg aerosolized ODN by
20 nebulizer.

 These results indicate that antisense oligonucleotides, even when modified to slow degradation, produce adenosine metabolites capable of potent bronchoconstriction when administered in asthmatic
25 airways.

 The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be
30 included therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Nyce, Jonathan W.
- (ii) TITLE OF INVENTION: Method of Treatment of Lung Diseases
Using Antisense Oligonucleotides
- (iii) NUMBER OF SEQUENCES: 44
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Kenneth D. Sibley
 - (B) STREET: Post Office Drawer 34009
 - (C) CITY: Charlotte
 - (D) STATE: NC
 - (E) COUNTRY: USA
 - (F) ZIP: 28234
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Sibley, Kenneth D.
 - (B) REGISTRATION NUMBER: 31,665
 - (C) REFERENCE/DOCKET NUMBER: 5218-32
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GATGGAGGGC GGCATGGCGG G

21

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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTAGCAGGCG GGGATGGGG C

21

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTGTTGGGC ATCTTGCC

18

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTACTTGCGG ATCTAGGC

18

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGGGCCTAG CTCTCGCC

18

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTCGGGGTAC CTGTCGGC

18

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGCTTTTCTT TTCTGGGCCT C

21

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCGCCGTGC CGCGTCTTGG TGGCGGCGG

29

- 48 -

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTCCCCTGG GTCTTCC

17

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCTGTGTCT CTCCTCCT

18

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCTTTCCTG GTTCTCTT

18

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

-49-

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCTGTGTCT GTCCTCCT

18

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCTCCCTTGG GCTCTGGCTC CTTCTC

26

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGCTCCTG GGGGCCTCCT G

21

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTGCTCCTG GGGGCCTCCT G

21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGTGCGGG GCCTGGTGCC

20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 15
- (D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGCGCGGGC GAGCATCGC

19

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
CCTCCTTCCT GGTCTGTCTG C 21
- (2) INFORMATION FOR SEQ ID NO:19:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
GCCCTGCTGC TCTTTCTGCT 20
- (2) INFORMATION FOR SEQ ID NO:20:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
GCGCTCGGCC TGGTCCCGG 19
- (2) INFORMATION FOR SEQ ID NO:21:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
GCCCGGGCCG GGGGCTGCTG GG 22

- 52 -

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCTCTTTTCT GTTTTCCC

19

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTTCTTGGCT TCTTCTGTC

19

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCTGCTGGT TTTCTGCCTT CTGCCC

26

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 41 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 53 -

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTTTCTCTTT CGCTTTCTTT TCGTCTCCTG TTCCTCCTTT T

41

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTCTGTCTTG TTCTGGTCCT TCGTGGGGCT CTG

33

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTCTGGTTGG CTCCTTC

18

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TCCCTGTTTC CCCCCTTT

18

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GCTTCTCTTT CGTCCCGGT GGGCTCG

27

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCTTGTGTGC TCTGCTGTCT CT

22

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGGTGGGGCT GGGGCTCCGG GGTCTCTGCC CCTCCGTGC

39

(2) INFORMATION FOR SEQ ID NO:32:

-55-

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTCCTTCTTG TCCGCTGCC

19

- (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTGGGGCCTG CTCTCCCGGC CTCCG

25

- (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGTCCTCAT GGCTGGGG

18

- (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- 56 -

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGGTCCTCAT GGCTGGGGTC

20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTCTTTGTTT CTGGGCTCGT GCC

23

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 14
- (D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTTCATGGTG GCTAGGTGGG GC

22

(2) INFORMATION FOR SEQ ID NO:38:

- 57 -

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 10
(D) OTHER INFORMATION: /standard_name= "Reduced A"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGGGTGGGTA GGCCGTGTCT GGGG

24

- (2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGGTTTCCTT TGCGGTC

17

- (2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GTGCTCCGGT GGCTTTTT

18

- (2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 58 -

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 6

(D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 10

(D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGTCCAGCCA TGGGTCTGGG

20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GCTGGTCCTC TGCTGTCCTT GCTG

24

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GCCCCGTCTG CTGCTCCTCG TGCCG

25

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 6

(D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 17

(D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTTTCATCTT GGCTTTATCC

20

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THAT WHICH IS CLAIMED IS:

1. A method of treating airway disease in a subject in need of such treatment, comprising:

topically administering an antisense oligonucleotide to the airway epithelium of said subject
5 in an amount effective to treat said disease;
said antisense oligonucleotide being essentially free of adenosine.

2. A method according to claim 1 wherein said airway disease is a lung disease and said airway
10 epithelium is a lung airway epithelium.

3. A method according to claim 1 wherein said antisense oligonucleotide comprises nucleotides in which at least one phosphodiester linkage is replaced with a linkage selected from the group consisting of
15 methylphosphonate linkages, phosphotriester linkages, phosphorothioate linkages, phosphorodithioate linkages, and phosphoramidate linkages.

4. A method according to claim 1 wherein said airway disease is selected from the group consisting of
20 cystic fibrosis, asthma, chronic obstructive pulmonary disease, bronchitis, and other airway diseases characterized by an inflammatory response.

5. A method according to claim 1 wherein said antisense oligonucleotide is targeted against an mRNA
25 encoding a protein selected from the group consisting of human A2a adenosine receptor, human A2b adenosine receptor, human IgE receptor β , human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D
30 synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion

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molecule-1 (ICAM-1), human vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor, human IL-3, human IL-4, human
5 IL-5, human IL-6, human IL-8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-alpha, human muscarinic
10 acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor α , human leukotriene C4 synthase, human major basic protein, and endothelin 1.

6. A method according to claim 1 wherein said
15 antisense oligonucleotide is delivered by administering an aerosol of respirable particles containing said antisense oligonucleotide to the lungs of said subject.

7. A method according to claim 6, wherein
said particles are selected from the group consisting of
20 solid particles and liquid particles.

8. A method according to claim 6, wherein
said aerosol is comprised of particles having a particle size within the range of about 0.5 to 10 microns.

9. A method according to claim 8 wherein said
25 particles are liposomes containing said antisense oligonucleotide.

10. A method according to claim 6 wherein said
antisense oligonucleotide is administered in amount sufficient to achieve intracellular concentrations of
30 said antisense oligonucleotide in said subject from about 0.1 to 10 μ M.

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11. A pharmaceutical composition, comprising,
together in a pharmaceutically acceptable carrier:

an antisense oligonucleotide in an amount
effective to treat an airway disease;

5 said antisense oligonucleotide being
essentially free of adenosine.

12. A pharmaceutical composition according to
claim 11 wherein said airway disease is a lung disease
and said airway epithelium is a lung airway epithelium.

10 13. A pharmaceutical composition according to
claim 11 wherein said antisense oligonucleotide comprises
nucleotides in which at least one phosphodiester linkage
is replaced with a linkage selected from the group
consisting of methylphosphonate linkages, phosphotriester
15 linkages, phosphorothioate linkages, phosphorodithioate
linkages, and phosphoramidate linkages.

14. A pharmaceutical composition according to
claim 11 wherein said airway disease is cystic fibrosis.

15. A pharmaceutical composition according to
20 claim 11 wherein said antisense oligonucleotide is
targeted against an mRNA encoding a protein selected from
the group consisting of human A2a adenosine receptor,
human A2b adenosine receptor, human IgE receptor β , human
Fc-epsilon receptor CD23 antigen, human histidine
25 decarboxylase, human beta tryptase, human tryptase-I,
human prostaglandin D synthase, human cyclooxygenase-2,
human eosinophil cationic protein, human eosinophil
derived neurotoxin, human eosinophil peroxidase, human
intercellular adhesion molecule-1 (ICAM-1), human
30 vascular cell adhesion molecule 1 (VCAM-1), human
endothelial leukocyte adhesion molecule (ELAM-1), human
P selectin, human endothelial monocyte activating factor,
human IL-3, human IL-4, human IL-5, human IL-6, human IL-

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8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-
5 alpha, human muscarinic acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor α , human leukotriene C4 synthase, and human major basic protein.

16. A pharmaceutical composition according to
10 claim 11 wherein said antisense oligonucleotide is delivered by administering an aerosol of respirable particles containing said antisense oligonucleotide to the lungs of said subject.

17. A pharmaceutical composition according to
15 claim 16, wherein said particles are selected from the group consisting of solid particles and liquid particles.

18. A pharmaceutical composition according to claim 16, wherein said aerosol is comprised of particles having a particle size within the range of about 0.5 to
20 10 microns.

19. A pharmaceutical composition according to claim 16 wherein said particles are liposomes containing said antisense oligonucleotide.

20. A pharmaceutical composition according to
25 claim 11 wherein said antisense oligonucleotide is administered in amount sufficient to achieve intracellular concentrations of said antisense oligonucleotide in said subject from about 0.1 to 10 μ M.

21. A pharmaceutical composition according to
30 claim 11, wherein said antisense oligonucleotide is conjugated to a molecule capable of cellular uptake.

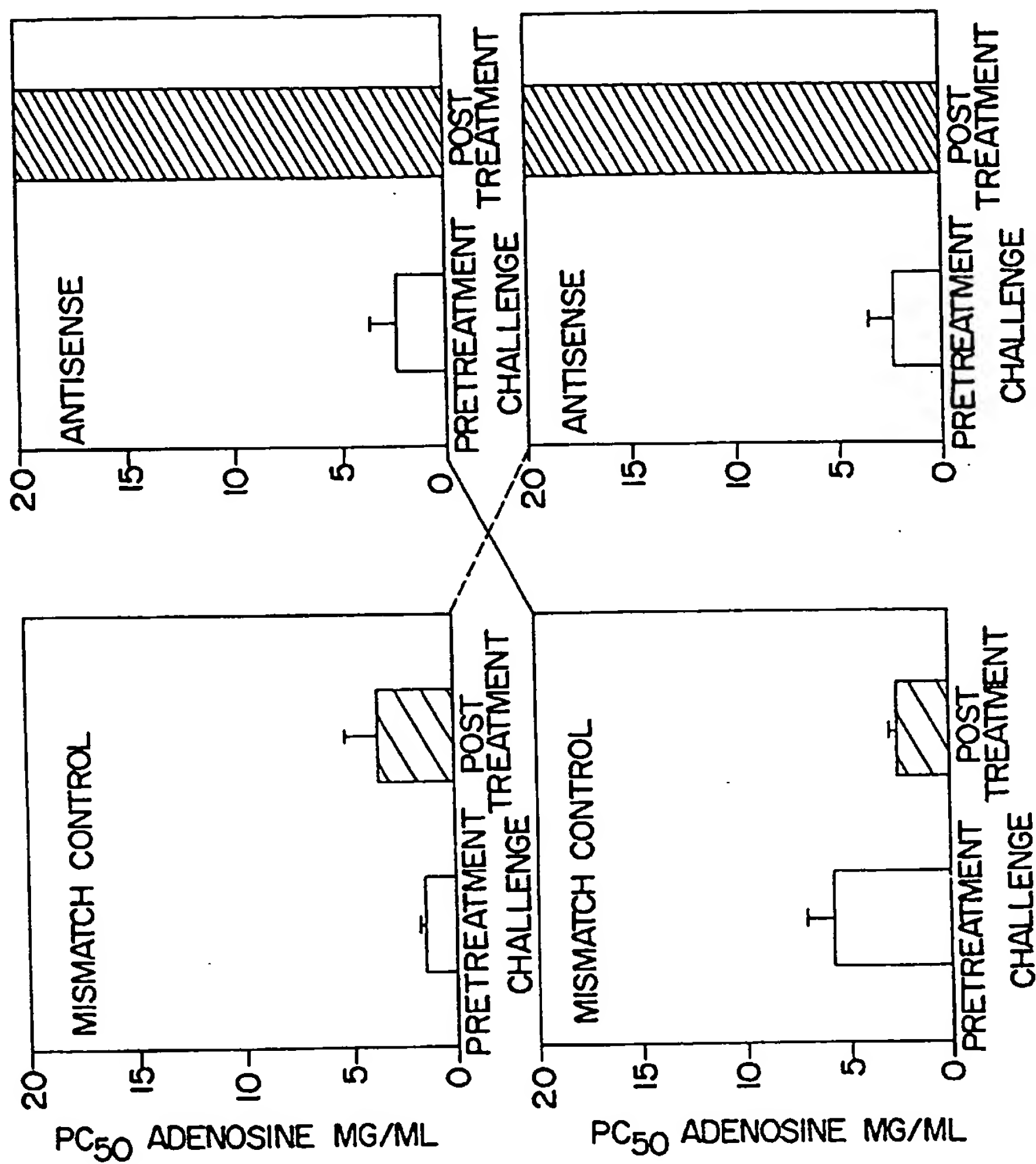
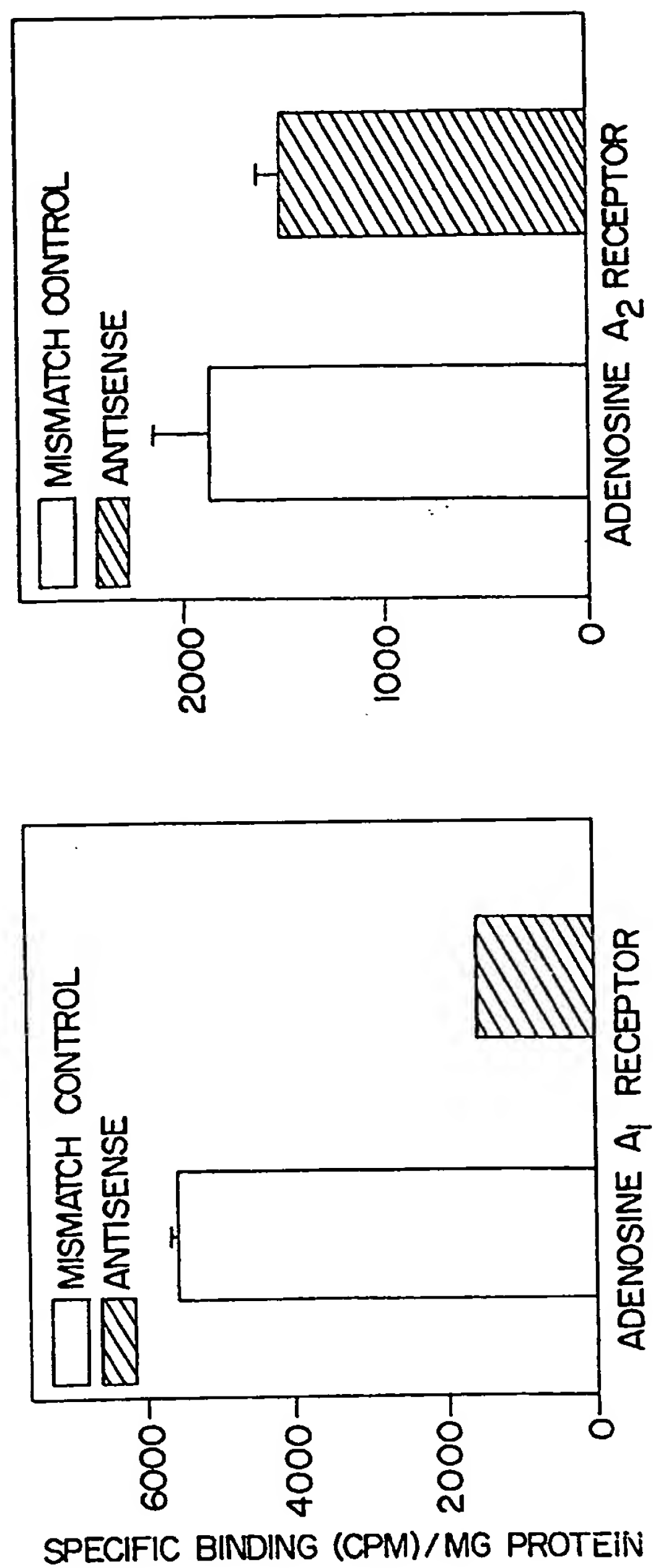
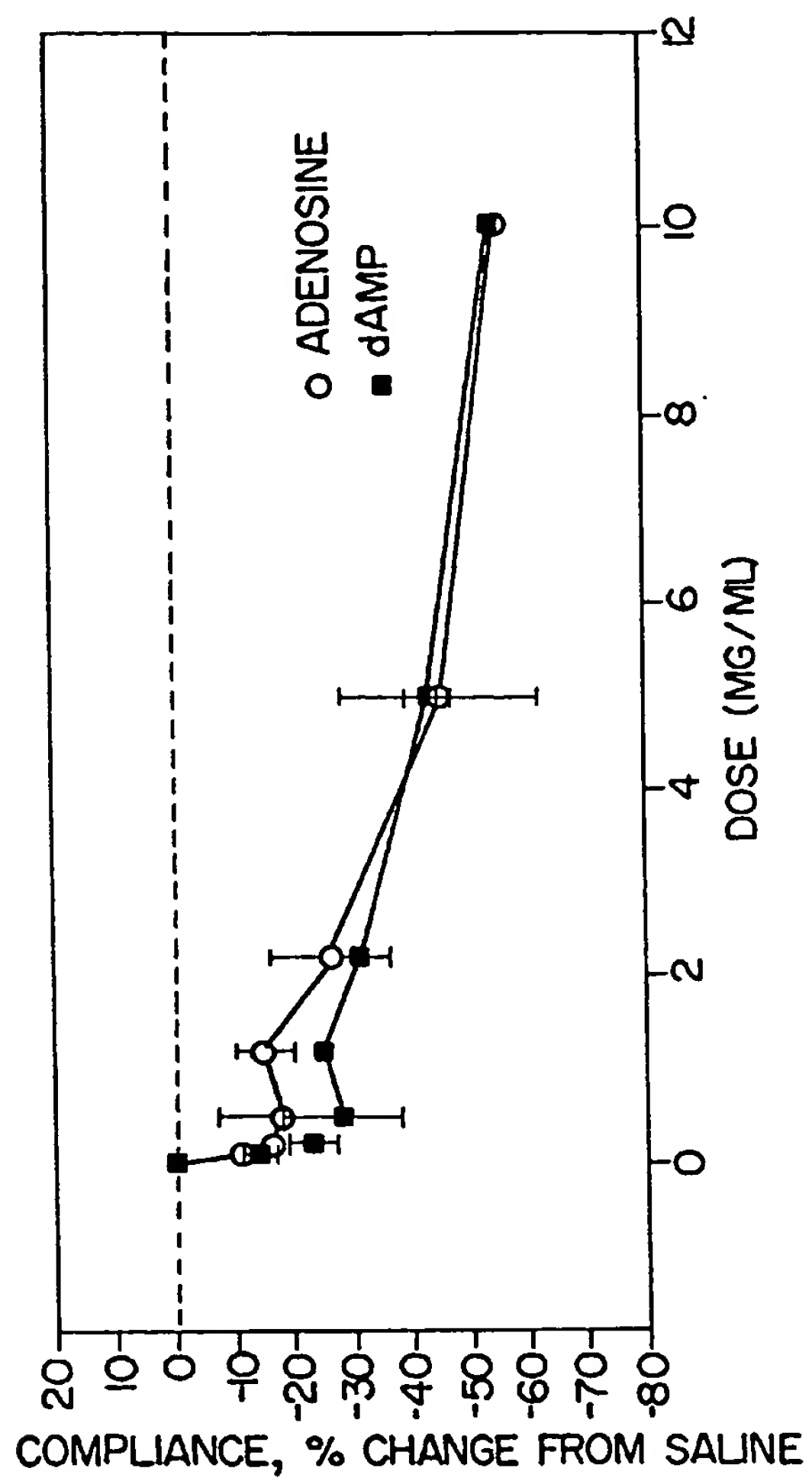


FIG. 1.

SUBSTITUTE SHEET (RULE 26)

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**FIG. 2.****SUBSTITUTE SHEET (RULE 26)**

FIG. 3.**SUBSTITUTE SHEET (RULE 26)**

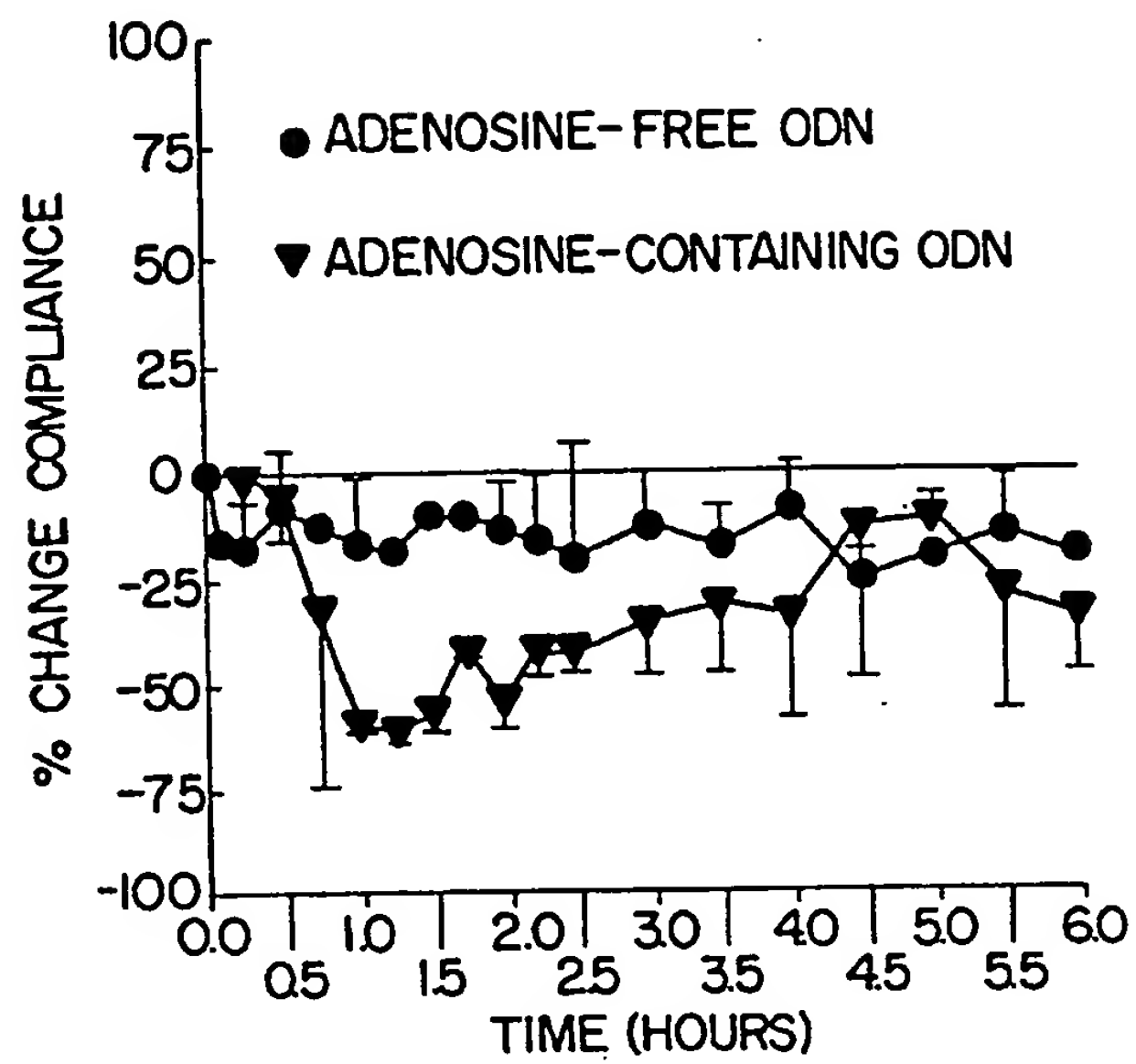


FIG. 4.

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09306

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70

US CL :514/44; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 5,514,788 A (BENNETT ET AL) 17 May 1993 (07.05.93), see entire document, especially Abstract, column 3, lines 15-18, column 5, lines 21-29, column 9, Figures 2 and 3.	1-6, 11-13, 15, 16 ----- 7-10, 14, 17-20, 21
X -- Y	WO 94/02605 A1 (DUKE UNIVERSITY) 03 February 1994 (03.02.94), see entire document, especially page 5, lines 9-15, page 18, line 28, page 20, lines 2-5, 11-15 and 31, page 21, lines 2-5.	1-4, 6, 7, 9, 11-14, 16, 17, 19 ----- 8, 10, 18, 20, 21
Y	US 5,264,618 A (FELGNER ET AL.) 23 November 1993 (23.11.93), see entire document, especially column 7, lines 40-42 and 54-56, column 8, lines 27-31, column 22, lines 12-15.	7-10, 17-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

18 AUGUST 1996

Date of mailing of the international search report

03 SEP 1996

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09306

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KNIGHT, V et al. Antiviral therapy with small particle aerosols. European Journal of Clinical Microbiology and Infectious Diseases. December 1988, Vol. 7, No. 6, pages 721-731, Abstract only.	7-10, 17-20
Y	SCHREIER, H. The new frontier: gene and oligonucleotide therapy. Pharmaceutica Acta Helvetiae. January 1994, Vol. 68, No. 3, pages 145-159, Abstract only.	14
Y	US 5,521,291 A (CURIEL ET AL.) 15 December 1993 (15.12.93), see entire document, especially column 13, lines 49-54, column 25, lines 17-19, 46-50, 50-62.	21

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*